FROM DESPAIR TO HOPE: STUDIES in HIV and TUBERCULOSIS 1992-2011

THESIS SUBMITTED FOR

THE DEGREE OF DOCTOR OF SCIENCE (Med)

IN THE UNIVERSITY OF CAPE TOWN

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To my mother Joyce Edna Wood who sacrificed much that I could receive the education she was denied.

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Introduction

This thesis is titled "From Despair to Hope" as these studies of HIV and AIDS were performed over a period when the prognosis for HIV-infected patients presenting with AIDS, evolved from one of rapid progression to death to one with a potential for decades of healthy life. The HIV pandemic reached South Africa later than its neighboring countries and developed rapidly in a country undergoing political upheaval. South Africa was totally unprepared for the onslaught of AIDS, which presented the greatest medical challenge in the country's history. My research and academic work have been inexorably linked to the response to the onslaught of the HIV and TB epidemics in South Africa.

There is an aphorism amongst epidemiologists that "to control an epidemic you must first know your epidemic". The selected work (100 of 309 peer reviewed articles and book chapters) highlights my attempts to better understand both the South African HIV and HIV-associated tuberculosis epidemics and to disseminate scientific information that could influence and direct national and global opinions and policies.

The Oxford dictionary provides a definition of "seminal" as having the properties of a seed; containing the possibility of future development. However, history is lived forwards but written in retrospect. We know the end before we consider the beginning and we can never wholly recapture what it was like to know the beginning only. The seminal nature of this work must therefore be viewed within a rapidly changing historical and geographical context of what was known and not known at the time. Current readers already know the ending. Therefore, the studies where possible, are presented chronologically with some description of the contemporary knowledge *status quo* to illustrate this work's contemporaneous relevance. Thus I hope to enable the reader to better view the seminal nature and the extent to which the work played a part in the evolving knowledge and response to the dreadful epidemic that engulfed South Africa.

My UCT career dates from 1986 when I exchanged the role of a general practitioner in Zambia for that of a medical registrar training post at Groote Schuur Hospital. During my training I was encouraged by senior physicians to publish case reports of unusual conditions among patients under my care. In my Master of Medicine thesis I explored the role of chromium valency underlying mortality and morbidity associated with use of chromium containing traditional medicines. In retrospect, I realise that this study marked a progression from reporting "what" was happening to endeavoring to understand "why" this was happening. It has been a desire to understand what is clinically observed that has motivated my subsequent research career. At the end of my clinical training, my mentor Professor Ralph Kirsch suggested that it was now time for me to acquire some experience in the laboratory.

In 1991, I accepted a research fellowship at Stanford University, CA, USA and it was here that my close association with the HIV epidemic really started. I was fortunate to be able to discuss my work with scientific leaders in the field such as Luc Montaner and Bob Gallo, the co-discoverers of HIV. These were desperate days for the San Francisco gay community, which was full of fear. However, this was accompanied by an incredible unity of purpose to confront and conquer this terrifying disease. It was here also that I had my first exposure to community activists, who while initially challenging to a medical scientist were subsequently to prove vital allies in the long fight against this scourge. I have a memory from that time of standing in the pulpit of the pink painted "Gay and Lesbian" church in the Castro section of San Francisco explaining intricacies of the studies we were recruiting. It was here also that I met people of extraordinary talent and courage; Elizabeth Glazer who while facing her own and her children's mortality was to go on to found the Elizabeth Glazer Pediatric AIDS Foundation, and Randy Shilts, the AIDS correspondent for the San Francisco Herald, who had documented the San Francisco HIV epidemic, Randy presented me with a copy of his recently published book "And the Band Played On. Politics, People and the AIDS Epidemic" with the inscription dated May 1991 " To Dr Robin Wood thanks for all you've done for me and for many, many others, keep caring". Randy died

of AIDS, February 17th and Elizabeth on December 3rd 1994. Death was a constant companion throughout these years.

In 1993, I returned to UCT to take up a position of lecturer in the Department of Medicine and Consultant Physician at the New Somerset Hospital. It was while at New Somerset Hospital that I headed the first HIV clinic in the Western Cape, developed the first South African HIV natural history cohort (Cape Town AIDS Cohort), introduced the first antiretroviral therapy (ART) within the public health sector, started the first community ART clinic in Gugulethu (Hannan Crusaid Centre), and initiated the first ART protocols to be used subsequently in the national roll-out in 2004.

In 2004 I became director of the Desmond Tutu HIV Centre (DTHC), a research unit within the Department of Medicine situated in the Institute of Infectious Disease and Molecular Medicine. With the wider availability of ART my research focus moved towards the interactions between HIV and tuberculosis, which in turn led to exploration of why tuberculosis control was failing. In order to better understand the population dynamics of TB in a South African township, I developed a community TB research site at Masiphumelele. Research at this site has incorporated mathematical modeling, molecular epidemiology and innovative interventions. The DTHC has maintained a prodigious scientific output over successive years by combining a community footprint of services with careful clinical and scientific observations.

The selected publications are largely based on observations and analyses of clinical data from; the Cape Town AIDS Cohort at the New Somerset Hospital, the Hannan Crusaid Gugulethu ART programme, the Masiphumelele community and the Desmond Tutu HIV Centre clinical trials unit. I am indebted to the patients and staff who have supported my work over many years. My publications have been highly cited by my peers (*vide infra*).

PUBLICATIONS SUMMARY:

Book chapters = 11

Articles published in peer reviewed journals = 299

Articles awaiting publication = 10

Articles accepted for publication = 309

Article citations = 7,845[‡].

Hirsch H-index = 50, (50 articles with >50 citations each)

Egghe G-index = 82, (81 articles with $>81^2$ or 6,734total citations)

[‡]Google Scholar citations on 14th November 2011.

ABRIDGED CURRICULUM VITAE

PERSONAL DETAILS:

NAME:	**ROBIN WOOD**
DATE OF BIRTH:	11th October, 1948
NATIONALITY:	South African
SA MEDICAL & DENTAL COUNCIL:	1986 Registration No. MP 282162
	1990 Specialist Internal Medicine
	2009 Specialist Infectious Diseases
GENERAL MEDICAL COUNCIL, UK:	1974 Registration No 1743096
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CURRENT APPOINTMENTS:

Professor of Medicine, Department of Medicine, Faculty of Health Sciences, University of Cape Town

Director of Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, UCT

Research Fellow, Centre of Excellence in Epidemiological Modeling and Analysis. University of Stellenbosch

Visiting Scientist, Harvard University School of Medicine. Boston, MA, USA

EDUCATION:

King Edward VI School, Birmingham, UK	1961-67
Guys Hospital Medical School, London	1968-70
Balliol College, Oxford University	1970-74
Royal Postgraduate Medical School, London	1977
Tropical Medical School, Liverpool University	1984
University of Cape Town Medical School, SA	1986-90
Stanford University Medical School, CA, USA	1990-92

ACADEMIC QUALIFICATIONS& AWARDS:

B.Sc. Biophysics (1st Class Hons), London University	1970
B.M.,B.Ch. Oxford University	1974
Fellow of the College of Physicians (S.A.)	1990
M. Med. University of Cape Town	1990
Diploma of Tropical Medicine & Hygiene, Liverpool University	1984
Diploma of Royal College of Obstetrics and Gynaecology, London	1982
Infectious Diseases Fellowship. Stanford University CA. USA	1992
Special Medicine Service Award, South African Medical Association	2008
Fellow of the University of Cape Town	2010
Lifetime Health Care Professional Award. Treatment Action Campaign	2010

PRIOR APPOINTMENTS:

2005-06 Professor of Medicine &Head of Division of Infectious Diseases. Groote Schuur Hospital, University of Cape Town (UCT)

2000-03 Associate Professor of Medicine (*ad hominem*)

1997-03 Principal Specialist and Head of Department of Medicine, New Somerset Hospital, Cape Town

1995-97 Senior Specialist Physician, New Somerset Hospital, Cape Town

1993-95 Specialist Physician, Head of HIV Clinic, New Somerset Hospital

1993-95 Lecturer in Medicine, Dept. of Med, UCT

1990-92 Clinical Fellowship in Infectious Diseases, Stanford University. CA, USA

1989-90 Senior Medical Resident, Renal Unit.Groote Schuur Hospital UCT

1986-88 Medical Resident, Dept. of Medicine, Groote Schuur Hospital, UCT

1979-85 General Practitioner & Medical Officer to US Embassy & British High Commission, Lusaka, Zambia

1982 Sept-Feb Senior House Officer. Obstetrics and Gynaecology. Poole General Hospital, Dorset, UK

1976-79 Senior House Officer. Medicine, Surgery, Orthopaedics and Paediatrics.Essex & West Kent A.H.A

1976 Feb-July Medical Officer. British Hindu-Kush Expedition, Afghanistan

1975-76 Senior House Officer. Emergency Unit. Radcliffe Infirmary, Oxford, UK.

1974-75 Intern. General Medicine & Surgery. Radcliffe Infirmary & Amersham, Berks

1973 June-Aug Student Medical Officer. Hospital Amazonico, Pucalpa, Peru

CURRENT DIRECTORSHIPS, COMMITTEES

Director, Desmond Tutu HIV Research Centre

Director, Cape Town Clinical Trials Unit

Director, Desmond Tutu HIV Foundation

University of Cape Town Faculty of Health Sciences, Bio-Ethics Committee Member

Member of the Governing council of the International AIDS Society

Scientific advisory board member, United States Government PEPFAR program

Scientific advisory board member, International Partnership for Microbicides

BOOK CHAPTERS:

1. Robin Wood. Contributing author. *Southern African Handbook of HIV Medicine*. Editors Wilson D and Bekker L-G. 1st edition, Oxford University Press Southern Africa, Cape Town 2002.
2. Robin Wood. Antiretroviral therapy. *HIV/AIDS in South Africa*, First Edition. Editors S S & Q Abdool Karim, Cambridge University Press, New York, USA 2005.
3. Robin Wood. Contributing author to Health System Response to the HIV Epidemic in South Africa**.** Editors Beck EJ, Mays N, Whiteside A, Zuniga JM. *The HIV Pandemic: local and global implications.* 2006 Oxford University Press, Great Clarendon Street, Oxford, OX2 6DP, UK.
4. Mauro Schechter, Robin Wood. Approach to the Management of HIV/AIDS in Developing Countries. In: *Sexually Transmitted Diseases*. Editor Piot P. 2008, McGraw-Hill, New York, USA.
5. Robin Wood, Des Martin. 2008. A decade of HAART in South Africa. In: Zuniga JM, Whiteside A, Ghaziani A, Bartlett JG, editors. *A Decade of HAART*. Oxford: Oxford University Press.
6. Robin Wood. Contributing author to Extra-pulmonary Tuberculosis. *Tuberculosis*. Editors Alimuddin Zumla and Simon Schaaf. 2008, Elsevier, London. UK.
7. Robin Wood. Large-scale implementation of antiretroviral therapy: Early results from faith-based clinics in South Africa. *Health Care in Rural South Africa*. Editors Adri Vermeer and Hugo Templeman. 2nd Edition, 2008, VU University Press, Netherlands.
8. Robin Wood. Contributing author. *Southern African Handbook of HIV Medicine*. Editors Wilson D and Bekker L-G.2nd edition, Oxford University Press Southern Africa, Cape Town 2008.
9. Robin Wood. Management strategies and therapeutic guidelines in resource-poor settings. In HIV/AIDS Editors Saleh El-Gadi and Brian Gazzard. Mediscript, London, UK. 2008.
10. Robin Wood. Antiretroviral therapy. *HIV/AIDS in South Africa*, Second Edition. Editors S S & Q Abdool Karim, Cambridge University Press, New York, USA 2008.

11. Steven Lawn, Robin Wood. Tuberculosis in HIV. In Infectious Diseases 3rd Edition. Editors Cohen and Powderly *et al*. Elsevier Health, USA 2010.

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13. Wood R, Hadden L. Malaria Prophylaxis for Expatriates. *British Medical Journal*. 1985;291:548.

PEER-REVIEWED ARTICLES: 1990-1994

14. Wood R. Benign Symmetric Lipomatosis: A Complication of Excessive Alcohol Consumption. *South African Medical Journal.* 1990;77(7):369-70.
15. Wood R, Mills PB, Knobel GJ, Hurlow WE. Acute Dichromate Poisoning After Use of Traditional Purgatives. *South African Medical Journal.* 1990;77(12):640-642.
16. Wood R, Commerford PJ, Rose AG, Tooke A. Reversible Catecholamine Induced Cardiomyopathy. *American Heart Journal.* 1991;121(2):610-613.[cit=54]
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Virus Infected Individuals. *Journal of Infectious Diseases*, 1994;170(4):1009-13.[cit=24]

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PEER-REVIEWED ARTICLES: 1995

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28. O'Keefe EA, Wood R. The impact of HIV on quality of life in a multiracial South African population. *Quality of Life Research*. 1996;5:275-280.[cit=59]
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70. Badri M, Ehrlich R, Pulerwitz T, Wood R, Maartens G. Tuberculosis should not be considered an AIDS-defining illness in areas with a high-tuberculosis prevalence. *International Journal of Tuberculosis and Lung Disease*. 2002;6(3):1-7. [cit=33]
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72. Boule A, Kenyon C, J Skordis, Wood R. Exploring of the costs of a limited public sector antiretroviral treatment program in South Africa. *South African Medical Journal.* 2002;92(10):811-817.
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75. Wood R. Therapeutic vaccines for individuals with HIV/AIDS (Review). *Continuing Medical Education Journal.* 2002;20(9):598-601.
76. Wood R. Effect of HAART on TB in South Africa. *Biomedicine & Pharmacotherapy.* 2002;56(8):423.

PEER-REVIEWED ARTICLES: 2003

77. Hoggard PG, Kewn S, Malherbe A, Wood R, LM Almond, SD Sales, J Gould, Y Lou, C De Vries, DJ Back, SH Khoo. Time-dependent changes in HIV nucleoside analogue phosphorylation and the effect of hydroxyurea. The effect of Hydroxyurea on nucleoside analogue phosphorylation (CHARM phosphorylation sub-study. *AIDS.* 2003;16(18):2439-46.
78. Badri M, Wood R. Usefulness of the total lymphocyte count in monitoring HAART in resource-limited settings. *AIDS.* 2003;17:541-545. [cit=74]
79. Orrell C, Bangsberg DR, Badri M, Wood R. Adherence is not a barrier to successful antiretroviral therapy in South Africa. *AIDS.* 2003;17(9):1369-75. [cit=212]
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PEER-REVIEWED ARTICLES: 2004

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Previously treated with Nelfinavir or Atazanavir. *Journal of Acquired Immune Deficiency Syndrome*. 2004;36(2):684-692. [cit=86]

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SELECTED PUBLICATION TOPICS

I. HIV-EPIDEMIC BEFORE ANTIRETROVIRAL THERAPY
   i. The beginning: AIDS studies in San Francisco
   ii. The dark ages
   iii. Cape Town AIDS cohort

II. ANTIRETROVIRAL THERAPY
   i. Is antiretroviral therapy feasible in South Africa?
   ii. Antiretroviral scale up
   iii. Operational studies
   iv. When to start

III. TUBERCULOSIS STUDIES
   i. Tuberculosis and HIV
   ii. Tuberculosis and antiretroviral therapy
   iii. Community transmission of tuberculosis

DETAILED SYNOPSIS OF THE CONTENTS OF THE WORK

I.HIV BEFORE ANTIRETROVIRAL THERAPY

The Beginning; AIDS studies in San Francisco [References 1-5].

As an introduction to my presented scientific work, I have chosen five papers from my time as an infectious disease fellow at Stanford University, which give some insight into the historical context, demonstrate the serendipity of scientific investigation and highlight the difficulty of identifying seminal works. Two papers illustrate an idea, which was the beginning of a great success story while the next three describe concepts that rapidly or eventually fizzled out. In the Journal of Acquired Immune Deficiency Syndrome [1] I reported early efforts to quantify HIV proviral DNA in different white cell populations. In the Journal of Clinical Microbiology, Jose Montoya and I went on to explore the relationship between quantity of provirus and immunological outcomes [2]. Together these papers report some early steps in an endeavor, which was the beginning of a great success story. Development of this nascent assay resulted in the licensure of a quantitative HIV viral load assay in 1999. Viral load was subsequently confirmed to be a predictor of HIV progression and is now in use by laboratories around the world. The ability to quantitate viral load has allowed identification of compounds with antiviral effect and viral load reduction is now an important component of antiretroviral drug development. However, a more negative measure of success has been the continuing legal battle over ownership and access to test royalties. The intellectual property dispute between Stanford University and the commercial developer reached the US Supreme Court in 2010.

The early nineties were a period of desperation as we had the tools to diagnose HIV infection and were able to measure the relentless progression of HIV disease but we had no effective treatments to stop that progression. The paper published in the Journal of Infectious Disease reflects the desperate search for treatments that could alleviate this progressively lethal disease [3]. The paper reports the results of an early strategy of giving long acting interleukin-2 (PEG IL-2), in order to reverse the immune deficiency known to accompany HIV disease progression. The treatment did temporarily increase CD4 cells but was very toxic. Two of my patients had serious neurological adverse events, which were fortunately reversible. I learned that IL-2 was also being used experimentally for the treatment of renal cell carcinoma at the National Cancer Institute (NCI). I therefore travelled across the USA to the NCI in Bethesda MA where we agreed to pool our experiences, which resulted in the collaborative publication in Journal of Neurology on the neurotoxicity of IL-2 [4]. The prospect of reversing immune suppression with IL-2 continued to have scientific appeal and studies of less toxic regimens of subcutaneous IL-2 continued. Unfortunately after 20 years several large studies have all failed to demonstrate any clinical benefit of immune based therapy with IL-2 strategy including the large STALWART (Study of Aldesleukin with and without Antiretroviral Therapy), SILCAT (Subcutaneous, Recombinant, Human Interleukin-2 in HIV-Infected Patients with Low CD4+ Counts under Active Antiretroviral Therapy) and ESPRIT (Evaluation of Subcutaneous Pro-leukin in A Randomized International Trial) studies.

The final paper from this era describes a study that I initiated, incorporated the pro-viral assay described above but was completed after my return to South Africa. The innovative but abortive strategy utilised a recombinant biologic chimeric protein combining domains of the CD4 molecule and *Pseudomonas* exotoxin A [5]. The concept was that the protein would bind to infected cells expressing gp120 and the exotoxin would kill the infected cell. Unfortunately binding of the chimeric protein to HIV-1 gp120 was probably impeded by the intense glycosylation ofgp120.

The Dark Ages [References 6-10]

I was fortunate to return to South Africa with some training in HIV management but I found the medical response to the epidemic was characterized by ignorance, fear, prejudice and fatalism. Care was encouraged outside of the medical sphere; patients were turned away from emergency units and denied hospital admission. Official policy was for terminal care to be encouraged away from the health care system, at home. This time has accurately been described as the "Dark Ages" replicating the mediaeval responses to the great plagues. Sick patients with AIDS were frequently turned away from health facilities and access to effective medications for readily treatable oesophageal candidiasis, chronic painful herpetic ulcerations and fungal meningitis were severely restricted.

Despite this environment, I continued the development of the first HIV clinic in the Western Cape at New Somerset Hospital with the resources and facilities available to me. We used the available resources to minimize the mortality, alleviate the morbidity and restore dignity to these social pariahs. Patients travelled from all over Cape Town to attend the HIV clinic where they were treated with dignity and compassion.

Medical need was swamping the resources of a single clinic and application of some rational triaging was outlined in a paper published in the journal AIDS in 1995 [6].

Until 1994 the "laying out" procedure for patients dying of AIDS in Cape provincial hospitals included sealing the body in double plastic bags and attaching "Danger of Infection" labels. The paper "HIV-infection: transmission hazards in life and death" was co-authored by the then professor of anatomical pathology and specifically targeted at the medical fraternity to highlight the rational observed risks rather than perceived risks of nosocomial HIV transmission [7].

The following paper documented the profound impact of HIV-infection on quality of life of our patients [8]. It was first necessary to develop a validated tool for measuring quality of life of the South African population in indigenous languages. When administered to HIV-infected patients stigma was shown to affect all studied population groups with a particularly marked decline in quality of life across a broad spectrum of functional and well-being measurements immediately after receiving an HIV diagnosis. In contrast, subsequent HIV progression affected quality of life by progressive erosion of physical functioning [9].

Monitoring of HIV disease progression was problematic as CD4 cell measurement was either not available or severely rationed. The accuracy of clinical staging was refined and in combination with the more readily available and cheaper total lymphocyte count was shown to be highly predictive for subsequent prognosis [10].

The Cape Town AIDS Cohort (References 11-21)

The HIV clinic at New Somerset Hospital allowed the development of one of few African natural history cohorts, the Cape Town Aids Cohort (CTAC). Clinical observations and laboratory data were collated and entered into a prospective electronic data capture system from 1992. Retrospective data from 1985 to 1992 was added by means of chart review. The spectrum of HIV-associated disease affecting USA and Europe had already been well described but there had been few descriptions from African cohorts. The change in HIV transmission in Cape Town from an earlier homosexual to later predominantly heterosexual epidemic was associated with changing clinical presentations in CTAC, which were described [11-13]. The associations between circulating HIV subtypes in patients infected by either homosexual or heterosexual transmission were also characterized for the first time [14]. These HIV-subtype analyses indicated that the HIV epidemic in South Africa had resulted from introduction of two major viral strains at different historical times and that these strains had to that data remained distinct.

CTAC allowed further definition of the natural history of HIV and AIDS in Cape Town. Articles were published on the aetiology of severe abdominal pain [15], the first descriptions of AIDS related disseminated histoplasmosis [16], the prevalence of toxoplasmosis infection in different South African racial groupings [17], and the spectrum and prognosis of AIDS presentations [18, 19]. The rate of progression of HIV infection [20] and short-term risk of AIDS and death were published in high impact international journals [21]. Much of this CTAC HIV natural history data was incorporated into the Harvard Medical School Cost-Effectiveness of Preventing AIDS Complications Model (CPAC) of HIV progression. The model had been initially developed for costing and efficacy projections for USA and has been the basis of ongoing collaboration with the Harvard team led by Kenneth Freedberg, to develop the CEPAC-International Model.

II. ANTIRETROVIRAL THERAPY

Feasibility of Antiretroviral Therapy in South Africa [References 22-28]

The prognosis for HIV-infected individuals in developed world settings dramatically improved following the presentation of results of combination antiretroviral therapy (ART) at the 1996 International AIDS Society Conference in Vancouver, Canada. AIDS changed from an inevitable rapid progression to death to a chronic disease capable of long-term management albeit at a high financial cost. With the exception of Brazil, which passed federal law 9313 for universal access to ART in December 1996, ART was restricted to wealthy countries. Somewhat incredibly the global *status quo* of no ART in the continent with the highest need was justified by prejudicial statements about inability of Africans to tell the time and take therapy appropriately. Therefore the national and international environment was not conducive for widespread use of ART. Furthermore, it was unclear when to initiate therapy, what drugs to use, how to monitor therapy and the costs and feasibility of large scale ART implementation. The first 3 papers were targeted at domestic policy makers arguing that an ART program was both feasible and affordable [22-24]. International concerns that

inevitable poor adherence of Africans would lead to ART resistance mayhem were addressed and demonstrated to be prejudicial and scientifically baseless [25]. Not surprisingly we showed that African patients were equally able as their western counterparts to take ART appropriately. The effectiveness of non-nucleoside regimens for initial ART was explored in the investigator initiated CHARM [26] and 2NN [27] studies. Participation in these international studies gained access for CTAC patients to ART and allowed us to construct the sequential treatment regimens, which were to later be used in the national ART roll out programme. The final paper used CTAC ART data to estimate the cost effectiveness of different ART implementation strategies [28]. This analysis was performed with the Harvard Medical School GAP model as described above.

Antiretroviral Scale Up [References 29-37]

In 2002 in partnership with the UK charity CRUSAID, we were able to initiate the first public sector community ART clinic in the Cape Town township of Gugulethu. Lessons learned during the first 6 months of this project were published in 2003 [29] followed by results of ongoing rapid scale up [30]. Feasibility of community ART provision was established, together with experience of the necessary staffing levels. A new cadre of therapeutic counselors was developed which increased community involvement and has subsequently continued to be deployed with in the expanded Western Cape ART programme. Analysis of the costs of the programme ensued [31, 32]. An interesting comparison between the results of this public health approach in Cape Town and those of individualized management available within the Swiss national cohort demonstrated similar clinical and virologic outcomes despite very different use of resources [33]. In 2004 the national department of health eventually commenced ART provision in the public sector using ART regimens, which had been developed in the Gugulethu project.

In collaboration with Ken Freedberg and Rochelle Walensky of Harvard Medical School, accumulated data from CTAC pre-ART and from our increasing cohorts on ART was used to populate the GAP model to project the results of different

scale up strategies on South African national mortality[34]. The need for patient management after treatment failure [35] and resources necessary to implement the recent changes in WHO guidelines were also modeled [36].

The South African HIV burden was such that doctor-led treatment could not cope with the necessary expansion of ART. I was principal investigator of the NIH funded first randomized trial of -nurse *versus* doctor-monitored ART (CIPRA 001) performed at Masiphumelele, which together with a parallel study in Soweto, Gauteng demonstrated that task-shifting from doctors to nurses was feasible strategy in South Africa [37]. The widespread provision of ART to our study population at Masiphumelele also enabled a second NIH CIPRA 003 study on the impact of ART provision on tuberculosis incidence and tuberculosis prevalence in a heavily burdened South African township (*vide infra*).

Monitoring an Antiretroviral Programme [References 38- 47]

ART access increased markedly in resource-poor countries with approximately 5 million patients on ART by 2010. The expansion of treatment access outstripped the capacity of laboratory services to monitor CD4 cell counts and viral loads. In order to address this problem, data was presented on the utility of the total lymphocyte count to predict CD4 cell response to ART [38], the utility of CD4 cell count to predict viral load [39], and the value of viral load [40]. I also developed the novel intelligent dispensing of ART (iDART) pharmacy-based programme monitoring tool in conjunction with the University of Cape Town engineering department [41] which has been widely used for monitoring ART programmes in South Africa and has subsequently been incorporated into the Western Cape "iKapa" and the proposed National HIV-ART monitoring systems. Despite initial concerns that "viral mayhem" could result from widespread use of ART, there has been little monitoring of genotypic resistance developing in predominant HI subtype C virus within the South African national progamme. However, we were able to describe the development of genotypic resistance after first-line and second-line ART in the Gugulethu cohort [42,43].

The South African public health approach HIV management has been based on the wide availability of a restricted number of cheap ART drugs. Conservation of ART [44] and substitution rates between regimens was described [45]. Much of the political opposition to the ART programme revolved around exaggeration of the toxicities of ART. However, we were able to demonstrate that ART generally improved quality of life and that in the minority with observed decline in quality of life, this was due in large part to HIV-related morbidities and not to drug toxicities [46].

While HIV prognosis is frequently described in patients receiving ART, the impact of ART provision at a population level is also determined by health seeking behavior and retention of patients in care. The carefully enumerated Masiphumelele community provided an almost unique opportunity to determine the population impact of an ART programme [47, 48].

When to Start Antiretroviral Therapy [References 49-63]

The threshold for starting ART has fluctuated widely since 1996 and has been likened to a swinging pendulum. In resource-rich countries, a "test and treat" policy followed observations of extremely high viral loads during asymptomatic HIV which was later replaced by a much more conservative approach following appreciation of long-term toxicities of the early ART regimens. Subsequent availability of less toxic ART together with the theoretical concept of treatment as prevention has led to a move towards early therapy again.

The decision of when to start ART is complex as the chosen threshold can be defined by either clinical or immunologic parameters [49]. The projected benefits of ART in the South African population are related to the differential mortality and morbidity with and without ART [50-55]and the CD4 cell response on ART at different initiation strategies[56]. The affordability and provision of ART may be restricted by locally relevant costs and cost-effectiveness [57, 58]. We are still awaiting the results of a controlled study of when to start (ongoing START study, for which the DTHC is the largest recruiter globally). In the

meantime, *post hoc* analyses from the SMART [59] and CIPRA 001 [60] studies that we conducted in Cape Town provide additional observational data informing when to start. Furthermore, reported data from the Gugulethu cohort demonstrated that generalized thresholds for initiating ART may need to be delayed or prioritized when co-infections are present [61-63].

III. Tuberculosis studies

Tuberculosis and HIV [References 64-78]

While the presentation of homosexually transmitted HIV-1 subtype B infection presented with a similar spectrum of opportunistic infections to that reported in Europe and North America, tuberculosis proved to be by far the commonest co-infection among heterosexually transmitted HIV subtype C. HIV infection changed the clinical presentations of tuberculosis and was described in the CTAC cohort [64-69]. The risk factors associated with development of tuberculosis were defined [70] and the impact on survival described [66].

The high mortality of TB co-infected patients required high levels of clinical suspicion and the development of new TB diagnostic algorithms [71-74]. The attributable-risk of HIV for TB disease [75] was such that there was a fundamental change in the age specific notification rates in a typical township population [76]. The tuberculosis control programme in Masiphumelele with its diagnostic emphasis on positive sputum-smear was less relevant for HIV co-infected patients and resulted in high prevalence of undiagnosed tuberculosis prevalence [77]. HIV-infection was shown to be radically changing the natural history of tuberculosis in the highly burdened Masiphumelele population [78].

Tuberculosis and ART [References 79-87]

It was recognized that ART use was associated with a decreased tuberculosis incidence rate and we suggested, in a 2001 South African Medical Journal opinion piece, that ART might be necessary to regain control of the tuberculosis

epidemic in South Africa [79]. Comparison between the ART and no ART CTAC cohorts allowed estimation of the magnitude of the decrease in incidence of tuberculosis, which was due to ART [80]. The therapeutic benefit was related to the clinical stage of disease and the CD4 cell count at ART initiation. However, immune restoration with ART was incomplete and patients remained at increased susceptibility to tuberculosis. Prolonged follow up of the Gugulethu ART cohort showed that ongoing tuberculosis incidence was largely determined by updated CD4 counts rather than solely on the initial CD4 value at ART commencement [81]. One of the concerns about simultaneous treatment with ART and tuberculosis treatment had been the development of immune restoration disease. Studies within the Gugulethu cohort allowed a distinction to be made between unmasking of prevalent TB and incident tuberculosis [82]. The need for integration of ART and TB control programmes was discussed [83, 84]. We postulated that increased survival resulting from ART together with increased susceptibility for tuberculosis disease might have a paradoxical impact on the population incidence of tuberculosis. However, repeated randomised cross-sectional population based tuberculosis surveys in Masiphumelele as part of the CIPRA 3 study, demonstrated a profound decrease in untreated tuberculosis prevalence following ART scale up [85]. Subsequent deceases in tuberculosis notifications rates with increasing population ART use were also shown for the first time [86]. This section ends as it started ten years earlier with further discussion of the role of widespread ART for control of tuberculosis [87].

Tuberculosis transmission [References 88-101]

While it has been recognized that the burden of TB has been amplified by HIV epidemic, it has been unclear whether HIV has changed the transmission dynamics within South African communities. Infection rates can be more readily measured in children and we demonstrated high annual risk of TB infection throughout childhood in the Masiphumelele township population [88]. Subsequently we demonstrated high and continued TB infection rate throughout adolescence and HIV-uninfected young adults in Masiphumelele and greater Cape Town [89, 90]. In young children transmission probabilities were

geographically related to adult smear positive cases living in the household and on the residential plot but not related to the HIV-status of source case [91]. Knowledge of transmission probabilities in households allowed estimation of transmission probabilities and environmental conditions prevalent in township shacks [92].

A court case brought against the minister of correctional services by a plaintiff who had contracted tuberculosis while a prisoner gave specific details of the conditions within the awaiting trial section of Pollsmoor prison. The detailed information afforded a unique opportunity to mathematically model the transmission probabilities and explore the necessary TB control measures that should be implemented [93].

Molecular epidemiology studies in Masiphumelele demonstrated multiple circulating strains, that there was no evidence that specific strains were strongly linked to HIV-status and that there was little nosocomial transmission among HIV-infected individuals on ART [94]. The interaction between HIV and TB was modeled mathematically for the first time in the Masiphumelele population [95]. The significance of the magnitude clustering of strains within a population were explored [96], and the nature of the contact groups [97]. As tuberculosis and other respiratory diseases are dependent on person-to-person transmission of pathogens the first population-based social interaction study was performed [98]. Daily contacts were shown to be highest and most age-assorted among adolescents a group with the highest new tuberculosis burden.

In 2009, approximately 30,000 cases of tuberculosis were notified in Cape Town of which 85% were HIV tested. In conjunction with actuarial HIV population estimates it was possible for the first time to calculate the age specific tuberculosis rates for HIV-infected and uninfected population of Cape Town [99]. The tuberculosis rates among young HIV-negative adults were similar to those recorded in the pre-chemotherapy era. Social mixing patterns, which play a role in the very high reported TB incidence rates, were described for the first time in a developing world setting [100]. The high TB infection and disease rates in both

HIV-infected and uninfected populations are an indication that the existing tuberculosis control programme is failing and new approached must be considered [101].

In late2011the WHO and Stop-TB initiative published an international roadmap for TB research, which highlighted for the first time an urgent priority for "identification of the biological, environmental, population-based and social drivers of transmission of *Mycobacterium tuberculosis*". The development of the Masiphumelele cohort and many of the above-presented publications had specifically addressed those highlighted "urgent priorities" over the preceding five years.

I. HIV-EPIDEMIC BEFORE ANTIRETROVIRAL THERAPY

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STATEMENT AFFIRMING THAT THIS IS THE ORIGINAL WORK OF THE APPLICANT. WHERE THE WORK IS PART OF COLLABORATION, THE OTHER CONTRIBUTING AUTHORS HAVE BEEN ACKNOWLEDGED.

Most of the work to which I have referred in this document was carried out by myself. When not undertaken personally by myself, students under my supervision or in collaborations performed it with colleagues.
The selected work presented is largely based on studies from three clinical cohorts, which I initiated and sustained. I designed and implemented the clinical and laboratory databases used for the individual analyses in these works and closely supervised the ongoing data collection systems and quality control. The important conceptual advances that have been achieved by the work I have presented here were my creation.

Professor Robin Wood. B.Sc, B.M., B.Ch, DTM&H, M.Med, FCP(SA).

Institute of Infectious Disease& Molecular Medicine, October 2011

STATEMENT THAT THE CANDIDATE HAS NOT SUBMITTED THIS WORK FOR AN EQUIVALENT DEGREE AT THIS OR ANY OTHER UNIVERSITY

I hereby affirm that I have not submitted this work for consideration for an equivalent degree at this or any other University.

Professor Robin Wood. B.Sc, B.M., B.Ch, DTM&H, M.Med, FCP(SA).

Institute of Infectious Disease & Molecular Medicine, October 2011

Safety and Efficacy of Polyethylene Glycol-Modified Interleukin-2 and Zidovudine in Human Immunodeficiency Virus Type 1 Infection: A Phase I/II Study

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Safety and Efficacy of Polyethylene Glycol–Modified Interleukin-2 and Zidovudine in Human Immunodeficiency Virus Type 1 Infection: A Phase I/II Study

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The safety and efficacy of combined therapy with polyethylene glycolated (PEG) interleukin (IL)-2 and zidovudine was assessed in 19 human immunodeficiency virus type 1 (HIV-1)–seropositive subjects in a phase I/II open-label dose-ranging study. During courses of three weekly infusions of PEG IL-2, dose-limiting side effects were seen at 5×10^6 IU/m^2 and reversible encephalopathy in 1 subject at 3×10^6 IU/m^2. Significant increases were seen in CD4 cell counts ($P < .01$), NK cell activity ($P < .05$), and HIV-specific cytotoxicity ($P < .01$). Virologic monitoring (quantitative DNA polymerase chain reaction and p24 antigen assay) showed no evidence of increased HIV activation. Patients with CD4 cells <200/mm^3 were entered into a chronic dosing phase. PEG IL-2 was given at 14-day intervals at doses of 10^6 IU/m^2 for 8 weeks and 3×10^6 IU/m^2 for up to 16 weeks, resulting in mean CD4 cell count elevations of 16% and 33%, respectively. PEG IL-2 appears to warrant further investigation, especially in subjects with CD4 cell counts <200/mm^3, to determine whether increased lymphocyte numbers will translate into improved clinical outcome.

Human immunodeficiency virus (HIV) infection is characterized by both a decline in circulating CD4 cells and the progression to an immunodeficient state, with decrease in cytotoxic lymphocyte responses against all major HIV proteins [1–5]. The risk of developing an opportunistic infection becomes significant when the CD4 lymphocyte count falls beneath 200/mm^3 and continues to increase as the CD4 cell count further declines [6]. Conventional therapy for HIV infection is aimed at diminishing viral replication [7] and providing prophylaxis against opportunistic infections [8].

Advances in recombinant genetic technology have enabled cloning of cDNA for human interleukin (IL)-2 into *Escherichia coli* to produce recombinant (r) IL-2 in therapeutic quantities [9]. Continuous infusions of rIL-2 together with zidovudine have been shown in HIV-infected individuals with CD4 cell counts >400/mm^3 to increase HIV-specific and nonspecific cytotoxic responses, to elevate CD4 cell counts [10], and to decrease mononuclear cell HIV proviral DNA [11].

Polyethylene glycol (PEG)–modified IL-2 (Chiron, Emeryville, CA) is produced by covalently binding PEG, a polymer with a molecular mass of 6 kDa, to the primary amino group of rIL-2. PEG IL-2 is supplied as a sterile lyophilized powder that is dissolved in saline before infusion. The specific activity as measured in a lymphocyte proliferation assay is about one-third that of nonmodified IL-2, and the biologic half-life is ~10 times that of nonmodified IL-2 with a median initial (α) half-life of 180 min and a final (β) half-life of 740 min [12, 13]. One milligram of PEG IL-2 is approximately equivalent to 6×10^6 IU. The rationale of the present study was to investigate whether intermittent infusions of PEG IL-2 could reproduce the potentially beneficial immunologic, viral, and T lymphocyte changes seen with continuous-infusion IL-2 [10] and to extend those observations to subjects with lower CD4 cell counts. The ability to deliver the drug by intermittent peripheral venous infusion rather than by continuous central venous infusion introduces the prospect of developing a practical chronic dosing regimen. As the lymphocytes of an activated immune system may be at increased risk of HIV infection [14], the reverse transcriptase inhibitor zidovudine was added to the regimen.

Methods

Trial design. We conducted an open-label dose-ranging study in two phases. Zidovudine, 500 mg/day in divided doses, was taken by all subjects for at least 8 weeks before initiation of and during the study. Seventeen subjects entered the first study

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Subjects were recruited from the AIDS Clinical Trials Unit, Stanford University School of Medicine; the study was approved by the Stanford University committee on the use of human subjects, and all subjects gave written informed consent.

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Table 1. Characteristics of HIV-infected patients at entry to study of PEG IL-2 and zidovudine treatment.

Subject	Age, years	CDC class	CD4 cell count/mm³*		p24 antigen
			Before therapy	After start of zidovudine	
1	41.3	II	396	349	−
2	40.5	II	508	504	−
3	37.0	II	394	374	−
4	41.9	II	635	683	−
5	33.1	II	424	416	+
6	30.0	II	388	495	−
7	42.6	III	461	448	−
8	46.5	IV-D	409	486	+
9	45.9	II	—	200	−
10	39.1	II	—	93	+
11	48.3	II	—	53	−
12	36.3	II	—	7	−
13	29.8	IV-C-2	—	67	−
14	35.5	II	—	207	−
15	44.4	IV-C-2	—	272	−
16	42.8	II	—	98	−
17	40.0	IV-D	—	51	+
18	41.3	II	—	385	−
19	39.9	V-C-2	—	297	−

NOTE. CDC, Center for Disease Control.
* Mean of ⩾3 counts.

phase, consisting of a series of 6-week cycles of three weekly infusions of PEG IL-2 followed by 3 weeks of zidovudine alone. Subjects received 1–3 courses consecutively, with a total of 41 courses of PEG IL-2 being given. The initial dose of PEG IL-2 was 3×10^6 IU/m², with dose escalation to establish the maximum tolerated dose and subsequent decrease in dosage to 10^6 IU/m².

In the second phase of the study, infusions were given at 14-day intervals for a 16-week chronic dosing regimen in which 5 subjects with CD4 cell counts of 200–400/mm³ received eight infusions of 10^6 IU/m² and 7 subjects with CD4 cell counts <200/mm³ received four infusions at 10^6 IU/m² and 5 subjects a further four infusions at 3×10^6 IU/m². Two subjects with CD4 cell counts <200/mm³ did not complete dosing at 3×10^6 IU/m² because they developed opportunistic infections and grade 3 hypotensive episodes. To confirm if the observed responses could be maintained, 3 subjects in each group continued dosing for a further 8 weeks.

Subjects. Individual subject profiles are given in table 1. Inclusion criteria included a Karnofsky performance status of >90%, hematocrit >30%, neutrophil count >1000/mm³, platelets >100,000/mm³, creatinine <1.5 times the upper limit of normal, transaminases <1.5 times the upper limit of normal, and no active opportunistic infection.

Toxicities. Clinical and laboratory toxicities were graded as grade 1, mild; grade 2, moderate, occasionally requiring treatment; grade 3, severe, requiring dose reduction or termination; or grade 4, life-threatening.

Blood cell separation. Peripheral blood mononuclear cells (PBMC) were obtained from fresh citrated venous blood by Ficoll-Paque (Pharmacia, Piscataway, NJ) centrifugation. Cells were removed from the interface, washed, and resuspended in RPMI 1640 (GIBCO, Grand Island, NY) with 10% fetal calf serum (Irvine Scientific, Santa Ana, CA).

Natural and lyphokine-activated killer cell assays. Briefly, K562 and Daudi tumor cells were labeled for 2 h with chromium 51 (Amersham, Arlington Heights, IL), washed twice with RPMI 1640, and coincubated with freshly isolated PBMC for 4 h in round-bottomed 96-well plates (Costar, Cambridge, MA) at 37°C and 5% CO_2. Assays were plated in quadruplicate at 10^5 labeled targets and PBMC effector-to-target (E:T) ratios of 50:1, 25:1, and 12.5:1 in a total volume of 200 μL. Cells were harvested (Skatron, Sterling, VA) and measured with a gamma counter (Gamma 5500; Beckman Instruments, Palo Alto, CA). Specific lysis was calculated as (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100.

HIV-specific cytotoxicity assays. Epstein-Barr virus–immortalized B cell lines were established and maintained as described [4]. These were then infected with recombinant vaccinia virus containing the bacterial (*E. coli*) β-galactosidase gene (Vac-*lacZ*) or the HIV *env*, *gag*, and *pol* genes (vPE16/Vac-*env*, VV:gag/Vac-*gag*, vCF21/Vac-*pol*, respectively; AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health [NIH], Bethesda, MD). Infection of immortalized B cells was done as described elsewhere [4]. These lymphoblastoid cell lines were used as targets for HIV-specific cytotoxic activities in a 6-h chromium 51 release assay. Autologous PBMC were used as effectors. E:T ratios used were 100:1, 50:1, 25:1, and 12.5:1. Monoclonal antibody to major histocompatibility complex (MHC) class I (PA2.6, a gift of A. M. Krensky, Stanford University Medical Center) was added at different concentrations at the beginning of the incubation period to detect the inhibition of MHC-restricted cytotoxicity. Specific cytotoxicity was calculated as (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100.

Proviral DNA quantification. This was a modification of the method described by Clark et al. [11]. Briefly, PBMC were frozen in RPMI 1640 with 10% dimethyl sulfoxide and fetal calf serum and stored at −180°C. Each thawed cell aliquot was centrifuged at 12,500 *g* in a microfuge for 5 min. The cell pellet was digested by overnight incubation with proteinase K at 55°C, followed by inactivation at 95°C for 10 min. DNA was extracted with phenol–chloroform–isoamyl alcohol (25:24:1), precipitated with 100% ethanol plus 0.3 *M* sodium acetate, and redissolved in 55 μL TE buffer (10 m*M* TRIS-HCl, pH 8.0, and 0.5 m*M* EDTA). DNA concentration was determined by spectrometer absorbance at 260 nm. At each time point, DNA was diluted in water to give 300,000, 100,000, and 30,000 cell equivalents at 6 pg of DNA/cell. Polymerase chain reaction (PCR) master mix, including primers SK39 and biotinylated SK38, was added to each sample, mineral oil was overlayered, and 40 cycles of amplification were done in a thermal cycler (model 480; Perkin-Elmer Cetus, Norwalk, CT). Samples were coamplified with a standard curve of dilutions of ACH2 cell DNA containing one HIV genome per cell (AIDS Research and Reference

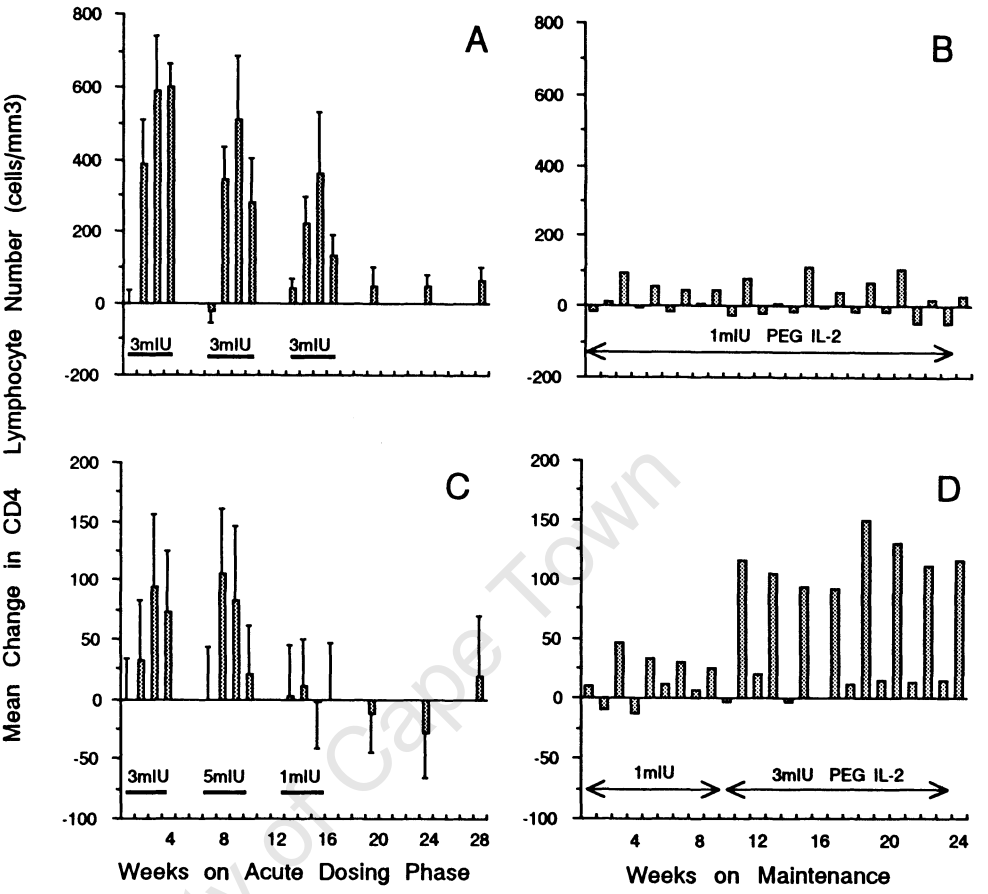

Figure 1. Mean (+SE) changes in circulating CD4 cell number from baseline values in four groups of subjects receiving PEG IL-2 during acute and chronic dosing phases. **A**, 6 subjects with baseline CD4 cell counts (mean) of 470/mm^3, during acute dosing phase. Three courses of three weekly infusions of PEG IL-2 (3×10^6 IU/m^2) were given at times shown by horizontal bars. **B**, 5 subjects with baseline CD4 counts (mean) of 391 cells/mm^3, who received biweekly infusions of 10^6 IU/m^2 at weeks 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23. Data from weeks 16–24 are of 4 subjects who continued maintenance for 24 weeks total. **C**, 3 subjects with baseline CD4 cell count (mean) of 115/mm^3, during acute dosing phase. Sequential courses of PEG IL-2 at 3, 5, and 1×10^6 IU/m^2 were given at times shown by horizontal bars. **D**, 5 subjects with baseline CD4 count (mean) of 144 cells/mm^3, who received biweekly infusions of PEG IL-2: 1×10^6 IU/m^2 was given at weeks 1, 3, 5, and 7; 3×10^6 IU/m^2 was given at weeks 9, 11, 13, 15, 17, 19, 21, and 23. Data from weeks 16–24 are of 3 subjects who continued maintenance for 24 weeks total.

Reagent Program, Division of AIDS, NIH) and negative and positive controls. Avidin-coated 96-well microplates were used to capture PCR product, and a horseradish peroxidase–labeled SK19 probe with a substrate of *o*-phenylenediamine was used to give a colorimetric readout [15]. Optical density was measured at 490 nm and was linearly related to input copy number between 10 and 3000 HIV copies, allowing calculation of sample HIV copy number by interpolation from the linear part of the standard curve. By using the CD4 cell percentage from the clinical flow cytometry laboratory, the HIV copy number was expressed per 1000 CD4 cells.

Serum p24 antigenemia. Serum p24 was measured with a commercial ELISA (Abbott, Abbott Park, IL) done and scored according to kit instructions.

Lymphocyte subsets. Lymphocyte subsets were determined immediately before and 7 days after each PEG IL-2 infusion; blood was drawn between 8:00 and 11:00 A.M. to avoid diurnal fluctuations. Absolute white blood cell counts were done on a cell counter (Baker, Phillipsburg, NJ). Lymphocyte subsets were determined by a whole blood lysis technique using fluorescein isothiocyanate– and phycoerythrin-labeled monoclonal antibodies (CD3, CD4, CD8, CD56, and Leu-M3; Becton-Dickinson, Mountain View, CA) and analyzed on an EPICS Profile II (Coulter, Hialeah, FL). Baseline CD4 cell values were established as the mean of three or more time points while the subject was on zidovudine during the 8 weeks before the commencement of PEG IL-2.

Statistics. For statistical analysis, CD4 cell numbers were expressed as logarithms ($\log_{10}$), changes in CD4 cell numbers as logarithmic changes, and means of CD4 cell numbers as geometric means. Analysis of variance (ANOVA) was used to compare the means of responses by different patient groups and dose regimens. Paired data were analyzed by two-tailed Student's *t* test. When trends from different dose levels appeared to be similar, data were pooled to obtain sufficient points for statistical analysis. Differences were considered significant at $P < .05$. For graphic clarity, changes in CD4 cell numbers were shown as absolute numbers.

Results

Effects on circulating T lymphocyte subsets. No significant changes in CD4 or CD8 cell counts were seen in the 5 subjects starting on zidovudine during the first 8 weeks of study. Six subjects completed three courses of 3×10^6 IU/m^2 PEG IL-2 (figure 1A). During each course, there was a mean elevation of 362–600 CD4 cells (72%–120% above baseline).

The peak response in each course followed the first or second of the three infusions, with no increased elevation following the third infusion. The CD8 cell responses paralleled the CD4 cell responses but showed greater variation with larger SEs. There was no significant change in CD4:CD8 ratio (data not shown); the changes in CD4 cell counts were attributable to the rise in total T lymphocyte counts. Three subjects received courses at each of the three dose levels; the CD4 cell count elevations were highest at 3 and 5 $\times$ 10^6 IU/m^2 and lowest at 10^6 IU/m^2 (figure 1C). Comparing the geometric mean of the three weekly CD4 cell counts while subjects were on PEG IL-2 with baseline values showed a 10% increase in circulating CD4 cell count with 10^6 IU/m^2, which, although statistically significant (geometric mean vs. baseline, $P < .05$), was of insufficient magnitude to be biologically significant. Statistically significant (geometric mean vs. baseline, $P < .01$) increases of greater magnitude (76%), more likely to be of biologic significance, were seen at 3 and 5 $\times$ 10^6 IU/m^2. ANOVA of the CD4 cell responses at each dose level demonstrated a very significant difference ($P < .01$) between 1 and 3 or 5 $\times$ 10^6 IU/m^2, but responses at 3 and 5 $\times$ 10^6 IU/m^2 were not significantly different.

Subjects in each clinical group responded to 3 $\times$ 10^6 IU/m^2. Group A subjects, with CD4 cell counts >400/mm^3, received 13 courses at 3 $\times$ 10^6 IU/m^2, with a mean increase in geometric mean CD4 cell number from baseline of 95% ($P < .01$). Group B subjects, with CD4 cell counts of 200–400/mm^3, received 6 courses at 3 $\times$ 10^6 IU/m^2, with a mean geometric increase of 37%. Group C subjects, with CD4 cell counts <200/mm^3, received 5 courses, with a mean geometric mean elevation of 74%. The results of groups B and C were combined for analysis and also reached statistical significance ($P < .01$). By ANOVA, the CD4 lymphocyte elevations of the three clinical groups were not significantly different.

Because of apparent cumulative toxicity and diminishing responses following the second and third weekly infusions, an alternate weekly schedule was used for maintenance. Seven group C subjects with a mean CD4 cell count of 144/mm^3 achieved a 16% elevation in geometric mean at 10^6 IU/m^2 for 8 weeks, and 5 subjects continued for 8 weeks at 3 $\times$ 10^6 IU/m^2 with a 33% elevation ($P < .01$ comparing geometric mean CD4 cell count while receiving PEG IL-2 with baseline values). Five group B subjects with a mean CD4 cell count of 391 achieved a 6% ($P < .05$) elevation of geometric mean for 16 weeks at 10^6 IU/m^2. These elevations were maintained by 3 subjects at each dose level for a further 8 weeks up to 24 weeks (figure 1B, D).

Lymphokine-activated killer (LAK) and NK cell activity. Our subjects' initial NK cell activity was on average lower than that in normal controls. During the first 8-week period of zidovudine treatment, there was a significant decline ($P < .05$ comparing mean values of weeks before and after starting zidovudine) both in NK cell activity and in circulating NK cells ($CD3^-/CD56^+$). After each course of PEG IL-2 therapy, there was a rise in NK cell activity (figure 2A), which after the second and third courses was statistically significant ($P < .05$ comparing mean values of the week before and after each PEG IL-2 course).

Infusions of PEG IL-2 at all dose levels studied (1, 3, and 5 $\times$ 10^6 IU/m^2) failed to produce any detectable LAK cell activity as defined by >10% specific lysis of Daudi targets at an E:T ratio of 50:1.

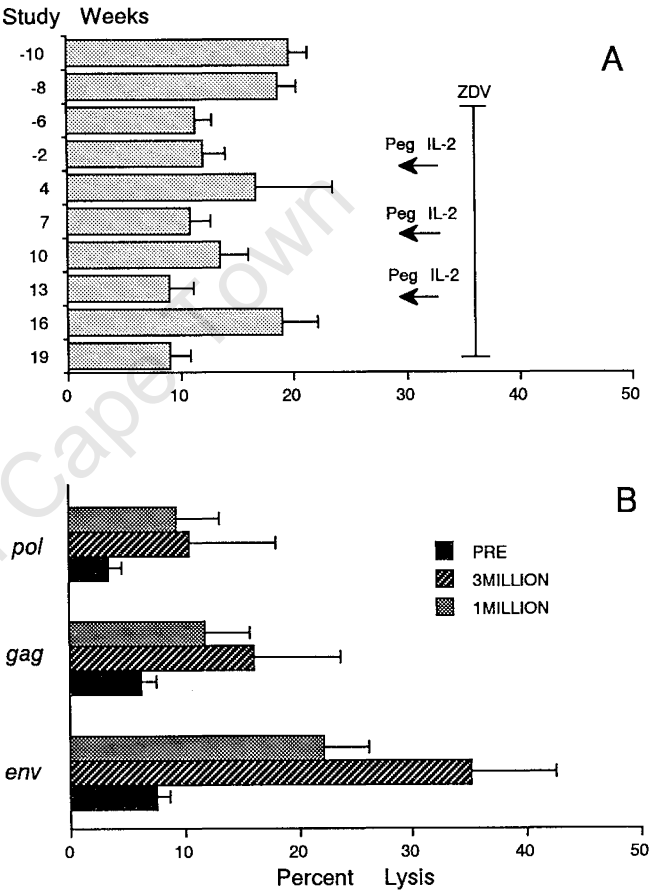

Figure 2. In vitro cytotoxic activity. **A**, NK cell activity of peripheral blood mononuclear cells (PBMC) of 8 subjects who started on zidovudine at beginning of study and completed three courses of PEG IL-2. Results are mean + SE of % K562 lysis at effector-to-target (E:T) ratio of 50:1. Fall in NK cell activity with zidovudine therapy and elevations after second and third courses were significant ($P < .05$). **B**, HIV major protein gene–specific cytotoxic activities of PBMC before and after PEG IL-2 therapy. HIV *env*-, *gag*-, and *pol*-specific cytotoxicities were obtained by subtracting % specific cytotoxicity of transformed B cells infected with vaccinia containing *lacZ* from that of transformed B cells infected with Vac-*env*, Vac-*gag*, and Vac-*pol*, respectively. Cytotoxicities of seven patients were measured before and after PEG IL-2 therapy. Three subjects received 10^6 IU/m^2, and 4 subjects received 3 $\times$ 10^6 IU/m^2. Each result is shown as mean + SE of % lysis of each group of patients at effector-to-target ratio of 50:1. HIV-specific cytotoxicities were significantly enhanced during 3 $\times$ 10^6 IU/m^2 PEG IL-2 therapy ($P < .01$).

Table 2. Log_{10} HIV provirus copies per 1000 CD4 cells of 7 subjects receiving three courses of 3×10^6 IU/m^2 PEG IL-2.

Subject	Week 0	Week 4	Week 10	Week 16
1	0.81	1.03	0.95	0.58
2	1.25	1.13	1.31	1.11
3	1.04	1.00	1.50	1.20
4	1.18	0.06	0.73	1.06
5	1.04	0.95	0.48	0.96
10	1.60	1.70	1.70*	1.70
11	2.68	2.57	2.66*	2.57
Mean	1.37	1.20	1.33	1.31

NOTE. Week 0, before PEG IL-2 on zidovudine alone; week 4, after course 1; week 10, after course 2; week 16, after course 3.
* PEG IL-2 dose, course 2, was 5×10^6 IU/m^2.

HIV-specific cytotoxicity. HIV *env*-, *gag*-, and *pol*-specific cytotoxicities increased after PEG IL-2 treatment. The enhancements of cytotoxicities were statistically significant ($P < .01$) after 3×10^6 IU/m^2 of PEG IL-2. The increase in cytotoxicities after 3×10^6 IU/m^2 was higher than that after 10^6 IU/m^2 (figure 2B). These cytotoxicities were HLA-restricted (data not shown).

PCR analysis of HIV provirus. Quantitative PCR analysis data are shown in table 2. There was no increase in mean proviral HIV DNA demonstrable after two courses of PEG IL-2 (weeks 4, 10, and 16).

Serum p24 antigenemia. Four subjects were p24-positive (>30 pg/mL) at the start of the trial (table 3). There was no significant increase in titers, and no one became positive during the trial period.

Toxicity. No patient discontinued or decreased zidovudine dosage because of adverse drug-related events. Toxicities encountered during the acute and maintenance PEG IL-2 phases are listed in table 4. Dose-limiting toxicities included severe fatigue, hypotension, neutropenia, and cryptic brachial plexopathy manifested by shoulder pain with subsequent winging of the scapula. All hypotensive episodes, defined as a drop in diastolic blood pressure >20 mm Hg, responded to bed rest and oral fluids (grade 2) or <500 mL of intravenous fluid (grade 3). The only grade 4 toxicity occurred in patient 7 (table 1), who became confused 60 h after the sixth PEG IL-2 infusion at 3×10^6 IU/m^2. Neurologic examination was compatible with a dominant parietal cortex lesion. There was rapid and full resolution of all neurologic signs and symptoms over the next 4 days. Lumbar puncture revealed a mild lymphocytic pleocytosis and a marginally elevated cerebrospinal fluid protein level. Magnetic resonance imaging (MRI) demonstrated multiple diffuse nonenhancing white and gray matter lesions that enhanced with gadolinium contrast material and slowly resolved over the following 8 weeks. This evolution of MRI changes was thought to be consistent with a vasculitic process but has not been confirmed by histology in any patient to date. This patient received no further PEG IL-2, and the subsequent weekly dose was reduced to 10^6 IU/m^2 for all subjects because of this central nervous system (CNS) toxicity and similar toxicity in two other PEG IL-2–treated cancer patients to be reported elsewhere. Two subjects (patients 10 and 11) developed cutaneous Kaposi's sarcoma during the 12-week

Table 3. Serum p24 core antigen levels in 4 subjects receiving PEG IL-2 and zidovudine.

Patient	Before	Week 0	Week 4	Week 10	Week 16	After
5	80	21	32	35	37	32
8	44	0	8	11	33	54
10	—	100	81	51	49	56
17*	—	225	330	—	—	251

NOTE. Subjects were initially positive for serum p24 (>30 pg/mL). Values are shown in pg/mL. Before, prior to starting zidovudine; week 0, after 8 weeks of zidovudine but before receiving PEG IL-2; week 4, after course 1; week 10, after course 2; week 16, after course 3; After, 1 month after PEG IL-2.
* Subject received only one course of PEG IL-2.

Table 4. Adverse events during PEG IL-2 therapy in HIV-infected subjects.

Symptom	Grade 1	Grade 2	Grade 3	Grade 4
Fever	16 (7)	1 (2)		
Myalgia	4 (3)	4 (6)		
Fatigue	3 (2)	— (6)	— (1)	
Nasal stuffiness	6 (1)			
Dizziness	1	— (2)		
Hypotension	—	1 (1)	2 (1)	
Neutropenia	—	1	2	
Dry skin	4 (5)	3		
Rash/pruritis	2 (2)	3		
Flushing	5 (2)	2 (1)		
Facial edema	6 (1)	1 (4)		
Conjunctivitis	1			
General edema	2 (2)	1		
Stomatitis	2 (2)	1		
Jaw pain	1	1		
Laryngitis	1			
Cough	3 (4)	— (1)		
Loss of appetite	1 (3)			
Nausea	6 (7)	— (2)		
Vomiting	1			
Loose Stool	4 (3)	— (3)		
Renal colic	1			
Headache	3 (5)	2 (1)		
Agitation	3 (1)	1		
Encephalopathy	—	—	—	1
Concentration problems	1 (2)			
Blurred vision	1 (1)			
Paresthesias	1 (2)			
Brachial neuritis	—	—	1	

NOTE. Data are no. of subjects out of 17 who entered acute dose-ranging phase (no. of subjects out of 13 who entered maintenance phase). Grade is maximum intensity of adverse event experienced.

interval between the two phases of this study. Subject 11 was removed from the study after diagnosis of cerebral toxoplasmosis after the fourth maintenance infusion. The maximum weekly tolerated dose of PEG IL-2 during the acute dosing phase, defined as the maximal dose at which <33% of subjects had grade 3 or 4 toxicities, was 3×10^6 IU/m^2.

Discussion

IL-2 is a pivotal cytokine of the cellular immune response, with a very short plasma half-life. Studies of bolus infusions of rIL-2 in HIV disease have shown variable immune enhancement [16–19]. A study of continuous-infusion rIL-2 in zidovudine-treated patients established an optimal daily dose at which there was an acceptable level of toxicity, associated with positive changes in immune parameters, including an increase in CD4 cell counts [10] and a decrease in mononuclear cell HIV proviral DNA [11].

PEG IL-2 is an analogue of IL-2 with a prolonged duration of action [19], which allows the drug to be given by intermittent peripheral venous infusion. Low-dose rIL-2 and PEG IL-2 have been given to HIV-infected patients by the subcutaneous route with immune enhancement but no elevation of lymphocyte counts [20]. The T lymphocyte responses to intravenous PEG IL-2 are complex. Peripheral T lymphocyte counts rapidly decline (data not shown) after intravenous infusion, reaching a nadir at 4 h, recovering to baseline values at 24–48 h, and rising to supranormal levels at 7 days. Elevations in circulating CD4 cells were seen after PEG IL-2 infusion in all patient groups defined by CD4 cell counts. The proportional elevation of CD4 cell counts ($\log_{10}$ changes or percentage rises) were similar in each group, although the absolute magnitude of these changes was baseline-dependent (i.e., was highest in group A and lowest in group C). Whether these new circulating cells represent an expanded T cell pool or a mobilization from the tissues to the circulation is not known at present. We did not assay soluble IL-2 receptors (sIL-2r), although Schwartz et al. [10] did show increasing sIL-2r levels peaking between 2 and 4 weeks of continuous rIL-2. The decreased CD4 lymphocyte response seen after the third weekly infusion may be modified by such an increase in sIL-2r.

One major concern with the use of cytokines to stimulate the immune system is that a fertile ground may be produced in which HIV will proliferate. Zidovudine was added to the regimen to inhibit viral replication, and both serum p24 levels and HIV provirus were assayed as virus markers. There was no overall change in either of these two measures during the study period. The decreases in HIV provirus reported by Clark et al. [11] following continuous rIL-2 were noted after the first initiation of both zidovudine and rIL-2 and may have been due to the zidovudine component.

Two subjects developed Kaposi's sarcoma between the two phases of this study. However, subject 8, who entered the study with cutaneous Kaposi's sarcoma, had partial resolution of his lesions over the course of the study.

Both the low initial NK cell activity of HIV-infected subjects and the fall with commencement of zidovudine therapy have been reported by Schwartz et al. [10]. The significant fall in NK cell activity with zidovudine in our study was associated with a fall in circulating $CD3^-/CD56^+$ NK effector cells (data not shown). The mechanism of this phenomenon is unknown, but as there was no associated decline in platelets, neutrophils, or red blood cells, it would seem unlikely that this was secondary to generalized zidovudine-induced marrow depression. The increase in both in vitro HIV-specific and NK cell activity following PEG IL-2 administration is compatible with the known immunomodulatory functions of IL-2 [21], but the clinical relevance of these changes remains to be determined. Our failure to demonstrate LAK cell activity 1 week after PEG IL-2 infusion is consistent with the findings of Schwartz et al. [10], who demonstrated LAK cell activity only once during IL-2 therapy, and Teppler et al. [20], who showed increased LAK cell activity with an assay requiring a 5-day preincubation with rIL-2.

The side effects of PEG IL-2 in our study were similar to those previously reported with the administration of IL-2 [22]. The lowest dose, 10^6 IU/m^2, was well tolerated, allowing administration on an outpatient basis. Higher doses, $\geq 3 \times 10^6$ IU/m^2, were associated with the risk of hypotension and necessitated observation of subjects for up to 12 h after infusion. Study of PEG IL-2 administration in animal models has demonstrated that effectiveness and toxicity are both dose- and interval-dependent [23]. Our clinical impression was that toxicities were cumulative when dosing was weekly, and our most serious toxicities occurred after high-dose weekly infusions. There appeared to be no cumulative toxicity with biweekly dosing.

Clinically significant CNS toxicities during IL-2 therapies are common, are often dose-limiting, and may progress after discontinuation of therapy [24, 25]. Two neurotoxicities developed during the weekly dosing phase of our study. The etiology of cryptic brachial plexopathy is often uncertain, although there may be an association with viral infections, including HIV infection. However, the temporal association of symptoms with PEG IL-2 infusion in our patient and the recent report of 2 cases of brachial plexopathy related to IL-2 therapy [26] would implicate PEG IL-2 as a probable causative factor. Both reversible encephalopathy [27] and acute fatal leukoencephalopathy [28] have been associated with IL-2 therapy, but the MRI lesions associated with neurologic symptoms in patient 7 have not previously been reported with IL-2 therapy. These lesions are compatible with a diffuse vascular phenomenon rather than a demyelinating process. Two further cases of subacute CNS toxicity with MRI scan changes have occurred during intense high-dose PEG IL-2 therapy (6×10^6 IU/m^2/week) for metastatic carcinoma (Yang JC, NIH personal communication). No further CNS

toxicity was observed during the maintenance phase of this study, in which the dosing interval was increased to alternate weekly infusions. Other side effects were not so limiting as to cause any patient to withdraw from the study.

The dose-ranging phase of this study demonstrated the maximum tolerated dose, toxicity, and potentially beneficial effects of PEG IL-2 therapy, while the maintenance phase showed that a tolerable and effective chronic dosing regimen with PEG IL-2 is possible. The combination of augmented immune function and elevation of CD4 cell count opens the possibility of therapeutically reversing two key markers of HIV progression. However, the dose of PEG IL-2 required to produce sufficient elevation of these parameters to be of biologic relevance is close to the maximum weekly tolerated dose. Subjects with CD4 cell counts $<200/mm^3$, because of their increased risk of opportunistic infections, may have more to gain from the dose-related CD4 elevations and augmented immune function seen in this study. The number of subjects in this pilot study was small, and further studies will be required, with larger groups of patients and controls, to ascertain whether manipulation of CD4 cells and cytotoxic responses by PEG IL-2 can translate into clinical benefit.

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Peripheral Blood Mononuclear Cell Human Immunodeficiency Virus Type 1 Proviral DNA Quantification by Polymerase Chain Reaction: Relationship to Immunodeficiency and Drug Effect

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Human immunodeficiency virus type 1 (HIV-1) proviral DNA from peripheral blood mononuclear cells (PBMCs) was quantitated in 61 HIV-1-seropositive individuals by a nonisotopic polymerase chain reaction assay. Primers from the *gag* region (SK38, SK39) were used to determine the $\log_{10}$ HIV-1 proviral copy number per 10^6 $CD4^+$ T lymphocytes (peripheral blood proviral load). A standard curve was generated for each assay by using ACH-2 cell DNA. The peripheral blood proviral load was followed in 15 individuals in a longitudinal study and was measured in 45 individuals in a cross-sectional analysis. Three of four untreated patients who were followed for 14 months had stable PBMC proviral loads and $CD4^+$ T lymphocyte counts; one untreated patient had a sustained increase in PBMC proviral load followed 5 months later by a significant decline in the $CD4^+$ T lymphocyte count. Eleven previously untreated individuals were monitored for 1 year following initiation of zidovudine and/or 2′,3′-dideoxyinosine therapy. The mean $\log_{10}$ number of proviral HIV-1 copies per 10^6 $CD4^+$ T cells decreased from 4.3 ± 0.4 at the baseline to 3.5 ± 0.6 after 2 to 4 months of therapy ($P < 0.01$). This initial 0.8 $\log_{10}$ fall in the PBMC proviral load after the initiation of therapy was followed by a rise in the PBMC proviral load by the sixth month of therapy. The PBMC proviral load in 45 subjects, both treated ($n = 25$) and untreated ($n = 20$), correlated inversely with the $CD4^+$ T lymphocyte count ($P < 0.01$, $R = 0.49$). PBMC proviral DNA quantification by a nonisotopic polymerase chain reaction assay correlates with HIV-1 disease progression and could be used to monitor the effect of antiretroviral therapy.

Quantification of human immunodeficiency virus type 1 (HIV-1) in peripheral blood by polymerase chain reaction (PCR) has been reported by using both RNA and DNA amplification (1, 5, 9–11, 13, 15, 17, 18, 21, 22, 24, 25, 27). Measurement of the HIV-1 proviral load may be useful in predicting the progression of disease and the response to currently available and experimental antiretroviral drugs (5, 8, 12, 15, 16). By using quantitative PCR, the amount of HIV-1 proviral DNA in peripheral blood mononuclear cells (PBMCs) has been shown to increase significantly and transiently at the time of symptomatic primary infection (6) and to rise gradually and steadily as the disease progresses (3, 9, 18, 24, 27).

PBMC proviral DNA amplification has also been used to assess the viral load response to therapy with immunomodulators or nucleoside compounds (4, 8). Both Clark et al. (4) and Aoki et al. (2) reported significant decreases in the amount of PBMC proviral DNA in drug-naive patients commencing zidovudine with recombinant interleukin-2 and 2′,3′-dideoxyinosine (ddI) therapy, respectively. However, Donovan et al. (7) found no significant change in the amount of PBMC proviral DNA in six patients after 5 to 14 months of zidovudine therapy.

Study of the peripheral blood proviral load ($\log_{10}$ HIV-1 copy number per 10^6 $CD4^+$ T lymphocytes), determined by a PBMC proviral DNA amplification assay developed and optimized in our laboratory, in 61 HIV-1-seropositive individuals is summarized here, as follows: a cross-sectional study of PBMC proviral load in 45 patients (25 patients on long-term therapy and 20 untreated patients) that correlated the PBMC proviral load with $CD4^+$ T cell counts and the impact of long-term antiretroviral therapy, a longitudinal study of the PBMC proviral load in 4 untreated patients who were followed for 14 to 16 months, and a longitudinal study of the PBMC proviral load in 11 previously untreated individuals starting single or combination nucleoside therapy.

MATERIALS AND METHODS

Specimens from 61 ambulatory and asymptomatic patients participating in AIDS Clinical Trials Group clinical trials at the Center for AIDS Research at Stanford University Medical Center in 1988 were available. All specimens were obtained from the patients with informed consent, and the study was approved by the institutional review board of the Stanford University Medical Center.

Citrated blood was obtained by peripheral venipuncture, and PBMCs were separated by Ficoll-Hypaque density gradient centrifugation. Cells were stored at −180°C. DNAs extracted from the PBMCs of seronegative individuals were used as negative controls in each assay. Cells were digested overnight at 55°C in proteinase K buffer, and proteinase K was inactivated by heating the samples at 95°C. DNA was extracted with phenol-chloroform (23), dissolved in 55 μl of TE buffer (10 mM Tris-HCl [pH 8.0], 0.5 mM EDTA), and quantitated by measuring the A_{260}. DNA equivalent to approximately 200,000 PBMCs (1.2 μg) per sample was

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amplified in a PCR mixture containing 15 mM Tris-HCl, 75 mM KCl, 2.5 mM $MgCl_2$, 0.4 μg of human placental DNA, 0.01% gelatin, 250 μM (each) deoxynucleoside triphosphates, 50 pmol each of the *gag* primer SK39 and biotinylated SK38 (20), and 2.5 U of *Taq* DNA polymerase (AmpliTaq DNA polymerase; Perkin-Elmer Cetus, Emerville, Calif.) in a total volume of 100 μl at pH 8.4. Amplification was carried out for 40 cycles in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus). Each cycle consisted of three steps of 30 s each at 94, 60, and 72°C, with a 10-min final extension step at 72°C.

PCR products were quantitated by using a colorimetric enzyme-linked immunosorbent assay format (14). Briefly, DNA was denatured at 95°C and rapidly cooled on ice, and 5 μl of PCR product from each sample was added to avidin-coated 96-well microplates. Hybridization solution containing horseradish peroxidase-labeled SK19 probe was added, and the plates were incubated at 42°C for 1 hour. By using a Biomek 1000 Automated Workstation (Beckman Instruments, Palo Alto, Calif.), each well was washed 20 times, *o*-phenylenediamine (horseradish peroxidase substrate) was added, and after adding 1 N H_2SO_4 to stop the reaction, the optical density at 490 nm was measured.

A half $\log_{10}$ dilution series of ACH-2 DNA containing one HIV-1 copy per genome (AIDS National Reagent Program, National Institutes of Health, Bethesda, Md.) was coamplified in duplicate with each PCR assay in order to establish a relationship between HIV-1 copy number and the optical density at 490 nm. The $\log_{10}$ of the optical density versus the $\log_{10}$ of the HIV-1 copy number generated a standard curve for each assay (14). This standard curve was linear between 10 and 1,000 copies of input DNA, with a mean R value for all standard curves of 0.98 and a standard error of 0.006. The HIV-1 copy number was calculated for each experimental sample by interpolation of the linear portion of the standard curve. HIV-1 copy number was normalized to 10^6 $CD4^+$ T lymphocytes by using the percentage of $CD4^+$ T lymphocytes in peripheral blood of the same experimental sample. The percentage of $CD4^+$ T lymphocytes in peripheral blood was measured independently by the Stanford University Flow Cytometry Laboratory by flow cytometry of total blood. The viral load was expressed as $\log_{10}$ HIV copy number per 10^6 $CD4^+$ T lymphocytes by the following formula: $\log_{10}$ HIV copy number/10^6 CD4 cells = PBMC $\log_{10}$ HIV copy number + $\log_{10}$ (100/percent CD4 cells). An analysis of HIV-1 proviral DNA amplification in lymphocyte subsets demonstrated that 93% of the HIV-1 proviral DNA was in the $CD3^+$-$CD4^+$ cell population. The contribution to the HIV-1 proviral DNA signal was minimal by the $CD45^+$-$CD20^+$ and the $CD45^+$-$CD14^+$ cell subsets (26).

Statistical analysis. Linear regression analysis between the half $\log_{10}$ dilution series of ACH-2 cell DNA and the optical density at 490 nm was performed by using the LINEAR program from the statistical package for Lotus 1-2-3 (Data Management Branch of the Division of Computer Research and Technology, National Institutes of Health). Student's t test for paired and unpaired data was used to compare the differences in the $\log_{10}$ HIV-1 copy number per 10^6 $CD4^+$ T lymphocytes within individuals over time and between groups of patients. Significance was defined as a P value of <0.05.

RESULTS

HIV-1 proviral DNA in peripheral blood was detected in 60 of 61 HIV-1-seropositive individuals. The number of copies of HIV-1 DNA detected in 2×10^5 PBMCs ranged between 40 and 200, which, if expressed as the number of infected $CD4^+$ T cells, is 10^3 to 10^5 copies of HIV-1 per 10^6 $CD4^+$ T cells. The only HIV-1 antibody-positive, PCR-negative sample was obtained from a patient with a CD4 count of 4. None of the DNA samples from 15 HIV-1-seronegative individuals was positive by PCR.

The PBMC proviral load, calculated as the $\log_{10}$ HIV-1 copy number per 10^6 $CD4^+$ T lymphocytes, in four untreated individuals with 190 to 270 $CD4^+$ T cells per mm^3 at the baseline measured monthly for 14 to 16 months is shown in Fig. 1. The viral load in three patients (Fig. 1A, B, and C) remained relatively constant over the period of the study; the mean difference between one determination and the next was 0.221 ± 0.163 $\log_{10}$ HIV-1 copies per 10^6 $CD4^+$ T lymphocytes. In these three patients, $CD4^+$ T lymphocyte counts remained relatively stable over the period of follow-up. In contrast, one patient (Fig. 1D) showed a 0.9 $\log_{10}$ sustained increase in viral load, from 3.8 to 4.7 HIV-1 copies per 10^6 $CD4^+$ T lymphocytes, which occurred between the fifth and seventh months of follow-up. This increase in viral load was followed by a sustained fall in the $CD4^+$ T lymphocyte count 5 months later.

To further evaluate the within-patient stability of the PBMC proviral load, the variability in measurements of the number of proviral DNA copies per 10^6 $CD4^+$ T lymphocytes was determined in paired samples from 14 patients. DNAs from paired separate aliquots of PBMCs obtained from 14 patients within 7 days before starting antiviral therapy were extracted, and assays for the number of copies of HIV-1 DNA were done. The mean difference between the 14 paired samples was 0.108 ± 0.089 (range, 0.04 to 0.275) $\log_{10}$ copy number per 10^6 $CD4^+$ T lymphocytes.

The PBMC proviral load was also determined in 20 untreated and 25 treated individuals (zidovudine [$n = 22$], ddI [$n = 2$], or dideoxycytosine [$n = 1$] monotherapy for more than 1 year) at a single point in time. The number of proviral DNA copies per 10^6 $CD4^+$ T cells increased with decreasing $CD4^+$ T lymphocyte counts ($R = 0.63$ and $P < 0.01$ for untreated individuals $R = 0.46$ and $P < 0.05$ for treated individuals).

To evaluate the effect of long-term antiviral therapy on PBMC proviral load, samples from the 45 patients were analyzed by comparing samples from untreated and nucleoside-treated (for more than 1 year) patients. Nucleoside-treated patients had a lower mean viral load (4.29 $\log_{10}$ HIV-1 copies per 10^6 $CD4^+$ T lymphocytes) than nucleoside-naive patients (4.49 $\log_{10}$ HIV-1 copies per 10^6 $CD4^+$ T lymphocytes). Although compatible with a drug effect, this difference did not achieve statistical significance.

PBMC proviral load was studied prospectively in 11 previously untreated patients before and after institution of nucleoside therapy. Seven patients were started on zidovudine or ddI monotherapy (Fig. 2A). Four patients were started on zidovudine and ddI in combination therapy (Fig. 2B). For 6 of the 11 patients, two baseline PBMC proviral load determinations were done preceding therapy; the mean difference between the paired baselines was 0.095 ± 0.106 $\log_{10}$. HIV-1 copies per 10^6 $CD4^+$ T lymphocytes. The mean PBMC proviral load in the monotherapy group at the baseline (4.4 ± 0.5 HIV-1 copies per 10^6 $CD4^+$ T lymphocytes [standard deviation; SD]) decreased to 3.8 ± 0.5 HIV-1 copies per 10^6 $CD4^+$ T lymphocytes at 2 to 4 months after the initiation of therapy ($P < 0.01$). Their mean CD4 cell count at the baseline of 215 ± 126 cells per mm^3 (SD) increased to 237 ± 103 cells per mm^3 (SD). The mean PBMC

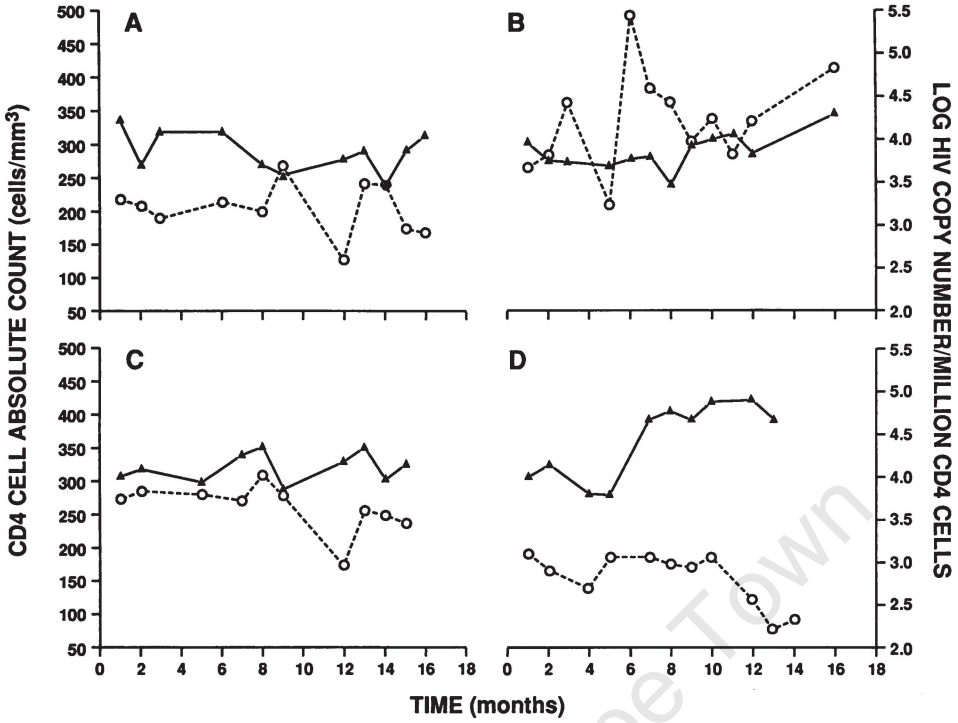

FIG. 1. Serial $CD4^+$ T lymphocyte counts (○) and $\log_{10}$ HIV-1 proviral copy number per 10^6 $CD4^+$ T lymphocytes (▲) in four asymptomatic individuals (A to D) who were followed for 16 months.

proviral load in the combination therapy group at baseline (4.17 ± 0.3 HIV-1 copies per 10^4 $CD4^+$ T lymphocytes [SD]) decreased to 2.96 ± 0.5 HIV-1 copies per 10^4 $CD4^+$ T lymphocytes (SD) at 2 to 4 months after the initiation of therapy ($P < 0.05$). Their mean CD4 cell count at the baseline of 360 ± 34 cells per mm^3 (SD) increased to 458 ± 52 cells per mm^3 (SD). In both the monotherapy and combination therapy groups, the increases in CD4 cell counts after therapy did not achieve statistical significance, as shown in Table 1.

DISCUSSION

Quantification of HIV-1 proviral DNA in PBMCs provides a direct measure of the number of infected cells in the peripheral circulation. The peripheral blood HIV-1 proviral load measured by the assay described here was stable over time in untreated individuals without progressive disease, correlated with the stage of disease as defined by the $CD4^+$ T lymphocyte count, and decreased following the initiation of therapy with nucleoside compounds in drug-naive patients.

To evaluate the stability of the signal over time, the PBMC proviral load was measured in four untreated individuals for a period of 14 to 16 months. These samples had been stored in dimethyl sulfoxide since 1988 and were analyzed in 1992. The $\log_{10}$ HIV-1 copy number per 10^6 $CD4^+$ T lymphocytes and $CD4^+$ T lymphocyte counts were stable over time in three patients; however, in one patient a significant decline in the $CD4^+$ T cell lymphocyte count was preceded by a 1-$\log_{10}$ rise in the PBMC proviral load. It appears that changes in the proportion of infected cells detected by this technique precede the subsequent deterioration of the immune system seen in HIV-1-infected individuals. A similar finding has been reported very recently by Connor et al. (5).

Several studies have shown a correlation between progressive disease and increasing copies of HIV-1 proviral DNA quantitated by PCR in sorted $CD4^+$ T cells (15, 24), PBMCs (3), or PBMCs corrected for the percentage of $CD4^+$ T cells in peripheral blood (9, 18, 19, 27). In the present study, the PBMC proviral load (expressed as $\log_{10}$ HIV-1 copy number per 10^6 $CD4^+$ T cells) increased with decreasing $CD4^+$ T cell count. However, a wide spectrum of PBMC proviral loads (up to a 100-fold range) was observed among patients with similar $CD4^+$ T lymphocyte counts. Further studies correlating the PBMC proviral load and clinical status in HIV-1-infected individuals are needed to determine whether PBMC proviral load qualifies as a better surrogate marker than $CD4^+$ T cell count of disease progression.

An important goal in the quantification of viral load in patients infected with HIV-1 is direct measurement of the impact of antiviral therapy. Although two studies have shown no significant change in the viral load in peripheral blood measured by proviral DNA amplification while patients have been on zidovudine (7, 19), in both of those investigations baseline measurements preceding the initiation of drug therapy were not obtained. In the study reported here, there was a modest but significant decrease in PBMC proviral load when baseline values were compared with those obtained 2 to 4 months after the initiation of therapy in drug-naive individuals. These findings are consistent with reports by Aoki et al. (2) and a previous study conducted in our laboratory (4) in which changes in PBMC proviral load were assessed in patients starting ddI and zidovudine therapy with recombinant interleukin-2, respectively.

after the initiation of therapy. $CD4^+$ T lymphocyte counts paralleled this fall, but its decline did not achieve statistical significance.

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Quantification and Comparison of HIV-1 Proviral Load in Peripheral Blood Mononuclear Cells and Isolated CD4$^+$ T Cells

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Summary: HIV proviral load was determined by quantitative DNA polymerase chain reaction (PCR) in peripheral blood mononuclear cells (PBMC) and lymphocyte subsets isolated by cell sorter. Provirus measured in PBMC, when expressed as HIV copy number per million CD4$^+$ cells, resulted in values which approximated those obtained from sorted CD4$^+$ T lymphocytes. A cross sectional analysis of HIV proviral load in CD4$^+$ T cells from 25 previously untreated and 30 zidovudine-treated seropositive patients with CD4$^+$ T-cell counts between 25 and 802/mm^3 demonstrated HIV copy numbers ranging from 1 copy per 10,000 cells in early disease to 1 copy per 10 cells in advanced disease. HIV proviral load can be rapidly assayed by PCR to give a reproducible value which varies over a 1,000-fold range and is positively correlated with cell infectivity as measured by a quantitative micrococulture assay. A less technically demanding assay using PBMC as substrate can give similar results to those obtained with sorted CD4$^+$ T cells. **Key Words:** HIV provirus—Quantitative PCR—CD4$^+$ T cells—PBMC.

Assays to quantitate HIV DNA by gene amplification have utilized both sorted CD4$^+$ T cells and peripheral blood mononuclear cells (PBMC) as substrates. As polymerase chain reaction (PCR)-based assays become more widely used in clinical trials, choices must be made concerning the most appropriate clinical samples to collect, store, and process. In this study, HIV proviral DNA from FACS-purified CD4$^+$ T cells was assayed at different stages of disease. In addition, the values obtained from sorted CD4$^+$ T cells were compared with viral load measured by a cell coculture assay and a simpler PCR assay using lysed PBMC as substrate.

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MATERIALS AND METHODS

Written, informed consent was obtained from all patients participating in this study who were volunteers screening for clinical trials between October, 1991 and March, 1992.

Cell Preparation and Storage

PBMCs were isolated from citrated blood by Ficoll-Hypaque centrifugation. Residual red cells were lysed with a solution of 0.84% ammonium chloride, 0.1% potassium bicarbonate, 0.2% sodium azide, 0.002% EDTA and the remaining cells washed twice in phosphate-buffered saline. One million cell pellets of PBMC were stored at −70°C. For FACS sorting, 5×10^6 PBMC were labeled with fluorescein-conjugated (FITC) anti-CD3 and phycoerythrin-conjugated (PE) anti-CD4 antibodies (Becton-Dickinson, Mountain View, CA, U.S.A.) according to manufacturer's instructions, washed and fixed in 0.5% paraformaldehyde. CD4$^+$/CD3$^+$ cells were isolated by a FACStarPLUS cell sorter (Becton-Dickinson, CA).

DNA PCR

The quantitative PCR assay used was a modification of the method described by Holodniy et al. (1). Cell pellets prepared as

described, were digested in 50 μl of 0.01% proteinase K buffer overnight at 55°C and then denatured at 95°C for 5 min. Fifty microliters of PCR reagent mixture was added to give final concentrations of 75 m*M* KCl, 3.75 m*M* $MgCl_2$, 15 m*M* Tris-HCl, 0.01% gelatin, 250 μ*M* of each deoxynucleoside triphosphate, 50 pmol each of HIV *gag* primers SK38 and biotinylated SK39 (Protein and Nucleic Acid Facility, Beckman Center, Stanford University) together with 0.6 μg of human placental DNA and 2.5 units AmpliTaq DNA polymerase (Perkin-Elmer Cetus) in each digested sample. These 100-μl reaction mixtures were amplified in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus) for thirty cycles with the following parameters: 95°C × 10 s, 55°C × 30 s, 72°C × 30 s. PCR product was quantitated by a nonisotopic method in which hybridization with an enzyme-linked probe allowed a colorimetric optical density readout. Ten microliters of denatured PCR product were added to avidin-coated 96-well microplates together with 65 μl of hybridization solution containing 1 pmol of horseradish peroxidase (HRP)-labeled SK19 HIV *gag*-specific probe and incubated at 42°C for 1 h. Plates were washed and 150 μl of HRP substrate containing *o*-phenylenediamine was added to each well. The reaction was stopped with 100 μl of 1 *N* H_2SO_4 and optical density of each well was measured at 490 nm. All the experiments were performed in duplicate or triplicate to confirm results. The HIV copy number of any sample was calculated by comparison of the resulting optical density with that of a coamplified half $\log_{10}$ dilution series of ACH2 (AIDS Research and Reference Reagent Program, National Institutes of Health, Bethesda, MD, U.S.A.) DNA. A linear relationship was obtained between the $\log_{10}$ of the optical density and input HIV DNA $\log_{10}$ copy number within the range 10 to 1,000 copies.

Microculture Assay

The microculture assay was performed according to the AIDS Clinical Trials Group standardized protocol (2). Briefly, five 5-fold dilutions of patient PBMCs were made from a starting concentration of 1.0×10^6 cells/ml. Each dilution was cocultured in duplicate in 24-well microculture plate with 1.0×10^6 phytohemagglutinin (PHA)-stimulated normal PBMCs. At 7 days half the medium was removed and replaced with fresh medium containing 5×10^5 PHA-stimulated PBMCs. Cultures were terminated on day 14 when a microculture well was scored as positive if >30 pg/ml of p24 antigen was present. Two consecutive negative wells defined an end point and split end points were scored as intermediate titers. For comparison with HIV provirus copy number, the limiting dilution titer was corrected for the CD4 percentage.

RESULTS

To explore the effect of varying the sample size within our assay, PBMC constructs with a fixed HIV copy number (ACH2 cells) were prepared and triplicate samples of 10,000, 30,000, and 100,000 cells were analyzed in the assay. For each of the constructs the optical density of the product was linearly related to the sample size (data not shown) demonstrating the ability to measure HIV copies in samples between 10,000 and 100,000 cells. The ability to combine a standard curve which was linear over a 100-fold range (10–10^3 copies) together with the adjustment of sample size between 10,000 and 100,000 cells enabled us to measure HIV copy numbers/million cells in any given subject over a 1,000-fold range (10^2–10^5 HIV copies/million cells).

The HIV log copy number/million $CD4^+$ T cells of 55 patients was plotted against their peripheral blood CD4 T cell counts at the time of venesection (Fig. 1). There was a significant correlation ($p = <0.001$) between increasing CD4 T-cell HIV load and decreasing $CD4^+$ T-cell counts.

The HIV log copy number/million $CD4^+$ T cells of 25 patients was plotted against the cell infectivity titer as determined by microculture assay (Fig. 2A), three other patients' PBMCs failed to grow in culture. There was a significant correlation ($p = <0.01$) between CD4 T-cell HIV load and culturable cell virus.

HIV log copy numbers from aliquots of lysed PBMC were compared with log copy numbers derived from analysis of aliquots of isolated $CD3^+$/$CD4^+$ cells (Fig. 2B). When the PBMC log copy number was corrected for the CD4 T-cell percentage [$+\log_{10}$ (100/CD4%)], similar results were obtained by both methods (mean difference 0.18).

DISCUSSION

The calculation of provirus copy number/million $CD4^+$ cells from assays performed on PBMC lysates is based on two assumptions, firstly, that the $CD4^+$ T cells are the major source of the HIV lym-

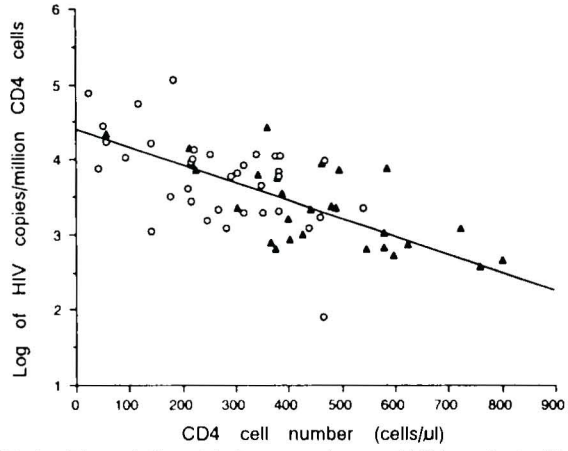

FIG. 1. The relationship between $\log_{10}$ of HIV copies/million $CD4^+$ cells of 55 patients and their CD4 T-cell counts at time of assay. The best fit regression line coefficient (*R*) was 0.61 ($p < 0.001$). ▲, untreated; ○, ZDV treated.

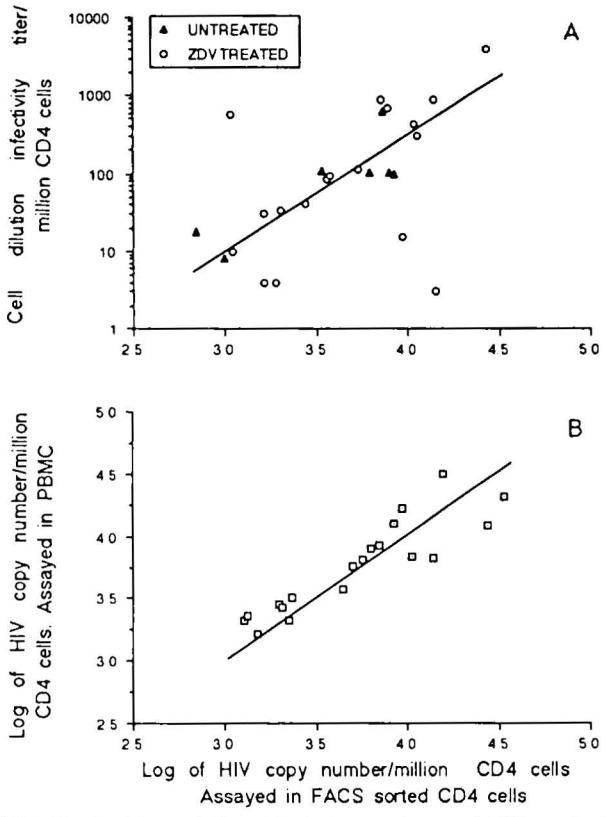

FIG. 2. **A:** The relationship between $\log_{10}$ of HIV copies/million CD4$^+$ cells of 55 patients and the cell infectivity titer/million CD4$^+$ cells. The best fit regression line coefficient (R) was 0.55 ($p < 0.01$). ▲, untreated; ○, ZDV treated. **B:** A comparison of $\log_{10}$ HIV copy number of 19 patients by both FACS-isolated CD4$^+$ T cells (abscissa) and with values from PBMC, but expressed per 10^6 CD4$^+$ T cells (ordinate) is shown. The results from both methods are closely related with an unforced R value of 0.904 ($p < 0.01$) an intercept of 0 and a slope of 1.

phocyte provirus and, secondly, that the CD4$^+$ T-cell percentage in the peripheral circulation represents the CD4$^+$ T-cell percentage in isolated PBMC. Several authors have reported CD4$^+$ T cells to be the major reservoir for HIV (3–5). An analysis of HIV proviral signal in lymphocyte subsets (CD3$^+$/CD4$^+$, CD3$^+$/CD8$^+$, CD45$^+$/CD20$^+$, and CD45$^+$/CD14$^+$ cells) demonstrated 93% of signal to be in the CD3$^+$/CD4$^+$ cell population (data not shown). Differing CD4 percentages in Ficoll-Hypaque and whole blood lysate samples have been reported in one study (6). Comparison in 10 subjects of CD4$^+$ T-cell percentages in both whole blood and isolated PBMC from the same venesection resulted in a mean error of 3.8% and a maximum error of 11%. This error is likely to be greater in those subjects with lower CD4$^+$ T-cell percentage.

Levels of HIV provirus of 1 in 1,000 to 1 in 10,000 CD4$^+$ cells in early disease are consistent with previous studies (3,7–9). In advanced disease, the presence of provirus DNA at levels of 1 in 10 CD4$^+$ cells is less certain. Studies utilizing in situ RNA hybridization (10) and DNA PCR (11) have demonstrated very low HIV expression and low provirus in PBMC of AIDS-related complex (ARC) and AIDS patients, whereas several other studies have demonstrated proviral loads comparable to those found in this study, i.e., 1 in 100 CD4$^+$ cells (3,7,9) and as high as 1 in 10 (8). Increase in viral load with disease progression has been previously demonstrated (8,9). The cross-sectional study of HIV-infected patients demonstrated that although there was a range of HIV DNA copy number at any given CD4 count, there was a statistically significant correlation between proviral copies/million CD4$^+$ cells and a decreasing CD4$^+$ T-cell count. The comparison of proviral copy number with infectivity titer demonstrated a positive association, although the number of culturable cells was between 10- and 100-fold less than the number of DNA copies. Explanations for this finding, include multiple proviral copies per cell, defective HIV DNA, nonintegration of HIV DNA, and latent nonexpressing provirus despite IL-2 stimulation in culture.

Quantitative PCR assays require accurate measurement of input substrate, which can be achieved by DNA extraction and quantification by spectroscopy or by counting input cell number. Quantification of cell number avoids a lengthy DNA extraction, but if frozen specimens are to be utilized, they must be enumerated before storage. Our CD4$^+$ T-cell assay utilized immunostaining and immediate cell sorting of fresh patient samples; the ability to perform such an assay is limited to relatively few centers. The assay of PBMC lysates gave similar results when CD4$^+$ T cells were enumerated at the time of venesection. This PBMC assay should allow the prospective collection of samples in large clinical trials to determine the short- and long-term effect of antiviral drugs on HIV provirus in CD4$^+$ cells.

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Multiple cerebral lesions complicating therapy with interleukin-2

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Article abstract—We reviewed the records and radiologic studies of eight patients who developed new focal neurologic abnormalities while receiving interleukin-2 (IL2)-based immunotherapy for malignancy or HIV infection. Initial confusion and delirium in the patients evolved into coma, ataxia, hemiparesis, seizures, and cortical syndromes including aphasia, apraxia, and cortical blindness. Imaging studies showed multiple white and gray matter lesions with a predilection for the occipital poles, centrum semiovale, and cerebellum. After cessation of IL2 treatment, seven patients improved to normal or near-normal neurologic function paralleled by resolution of the lesions on scans. One patient improved only minimally. Possible etiologies for the lesions include an IL2-induced cerebral vasculopathy, a direct toxic effect of IL2, or immunologically mediated damage.

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Interleukin-2 (IL2) is a lymphokine effective against advanced metastatic malignancies in some patients,[1-3] which may also act against the human immunodeficiency virus.[4,5] IL2 is used alone, in a long-acting preparation conjugated to polyethylene glycol (PEG-IL2), in combination with activated lymphocytes as part of adoptive immunotherapy, or with other cytokines such as alpha-interferon (alpha-IFN) or granulocyte-macrophage colony stimulating factor (GM-CSF).[1,6]

Systemic side effects of IL2 such as fever, chills, malaise, nausea, vomiting, and diarrhea are common. Fluid extravasation from a capillary leak syndrome may lead to hypotension, multiorgan failure, and arrhythmias.[1,7] The most common neurologic side effect of IL2 therapy is a reversible encephalopathy characterized by confusion, lethargy, and poor concentration.[8-10]

It can progress to delirium with psychotic features or to coma. Over the past several years, there have been a few case reports of focal neurologic deficits in patients receiving IL2. Aphasia, hemiparesis, seizures, visual distortions, and ataxia have occurred as well as brachial plexopathy and carpal tunnel syndrome.[11-17] Vecht et al.[16] reported an acute fatal demyelinating leukoencephalopathy in a patient receiving IL2.

As therapy with IL2 has become more widespread, neurologic illness has become an important cause of morbidity and a treatment-limiting complication. We describe eight patients who developed neurologic deficits and lesions on brain imaging during IL2-based treatment of malignancy or HIV infection.

Methods. From 1985–1994, over 1,500 patients received IL2-based therapy for malignancy at our institution, in-

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cluding five who developed neurologic illness with abnormalities on magnetic resonance imaging (MRI) during or shortly after treatment. No patient had metastases to the nervous system or a history of neurologic disease or stroke prior to receiving IL2. The medical records and imaging studies were reviewed. Three additional patients (cases 4, 7, and 8) receiving IL2 for HIV infection or malignancy at other institutions with similar neurologic symptoms, courses, and imaging studies were also studied.

Case reports. *Patient 1.* A 53-year-old man with renal cell carcinoma underwent nephrectomy and radiotherapy before referral for IL2. His immunotherapy included alpha-IFN and IL2. Five days after the start of treatment, the patient became progressively confused and lethargic and lapsed into coma. He had intermittent left focal seizures, which stopped spontaneously within 24 hours. Peritoneal dialysis was begun on the 10th hospital day for renal failure. Two days after beginning dialysis, the patient was alert but disoriented with hallucinations. The symptoms improved over the next week. On the 22nd hospital day, left focal seizures recurred and were followed by a generalized seizure. He was started on phenytoin.

Following the seizure, he had a left spastic hemiparesis. Cerebrospinal fluid (CSF) was normal except for a slight elevation in protein to 56 mg/dL. An electroencephalogram (EEG) showed diffuse background slowing with right temporal lobe periodic epileptiform discharges. A CT of the head showed nonenhancing white matter lucencies extending from occipital areas forward into the centrum semiovale. White matter hyperintensities, which were especially prominent occipitally, were seen on MRI.

He improved rapidly and was fully awake 48 hours after the seizure. A repeat MRI 3 weeks later was unchanged, and an EEG was improved. On discharge from the hospital on the 38th hospital day, the patient had persistent reflex asymmetry but an otherwise normal neurologic examination. MRI 1 month after discharge showed resolution of the white matter abnormalities.

Patient 2. A 41-year-old man with renal cell carcinoma started IL2 2 months after a radical nephrectomy. He received seven doses of IL2, then three weekly doses of PEG-IL2. During the second week of PEG-IL2 treatment, he developed right proximal arm weakness, sensory loss, and shoulder pain with winging of the scapula. Deep tendon reflexes were normal. MRI of the cervical spine showed bony degeneration and bulging disks at C-5 through C-7. MRI of the head showed a few small areas of high signal in the cerebral white matter.

A second cycle of therapy began 1 month later. He received five doses of IL2 followed by PEG-IL2 for 3 weeks. Two days after the last PEG-IL2 dose, he became delirious and was treated with haloperidol. On examination, the patient was lethargic but able to state his name and open his eyes on command. There was no visual fixation or response to visual threat. The pupils were small and reactive to light. A right gaze preference was present. Both legs moved spontaneously, but the right arm was immobile and hyperreflexic. The left arm withdrew to noxious stimuli only. Plantar responses were extensor. The patient had a generalized tonic-clonic seizure, which was treated with phenytoin.

At the time of neurologic deterioration, the white blood cell (WBC) count was 23,000/mm^3 with 46% eosinophils. Coagulation studies, serum sodium, magnesium, calcium, and liver function tests were normal. There was mild renal insufficiency. CK was markedly elevated at 1,024 U/L. A nonenhanced CT of the head showed no lesions. MRI showed new cortical, subcortical, and cerebellar lesions, including bilateral occipital lesions that did not enhance with gadolinium (figure 1A). The CSF had 3 white blood cells and 44 red blood cells/mm^3. CSF protein, glucose, and cultures were normal. An EEG done 4 days after the seizure showed diffuse slowing without epileptiform activity.

The patient remained intubated and pharmacologically paralyzed for 5 days after the seizure. When paralysis and sedation were discontinued, he gradually awoke. He was able to read and identify objects and colors, but complained of difficulty locating objects visually. The right arm remained weak and atrophic. MRI of the head 1 week later showed persistence of the brain lesions, which now enhanced with gadolinium (figure 1B).

Two months later, his mental status and vision were completely normal. The right arm weakness had improved. An MRI 5 months after admission showed only those small white matter lesions that had been present prior to the second cycle of IL2.

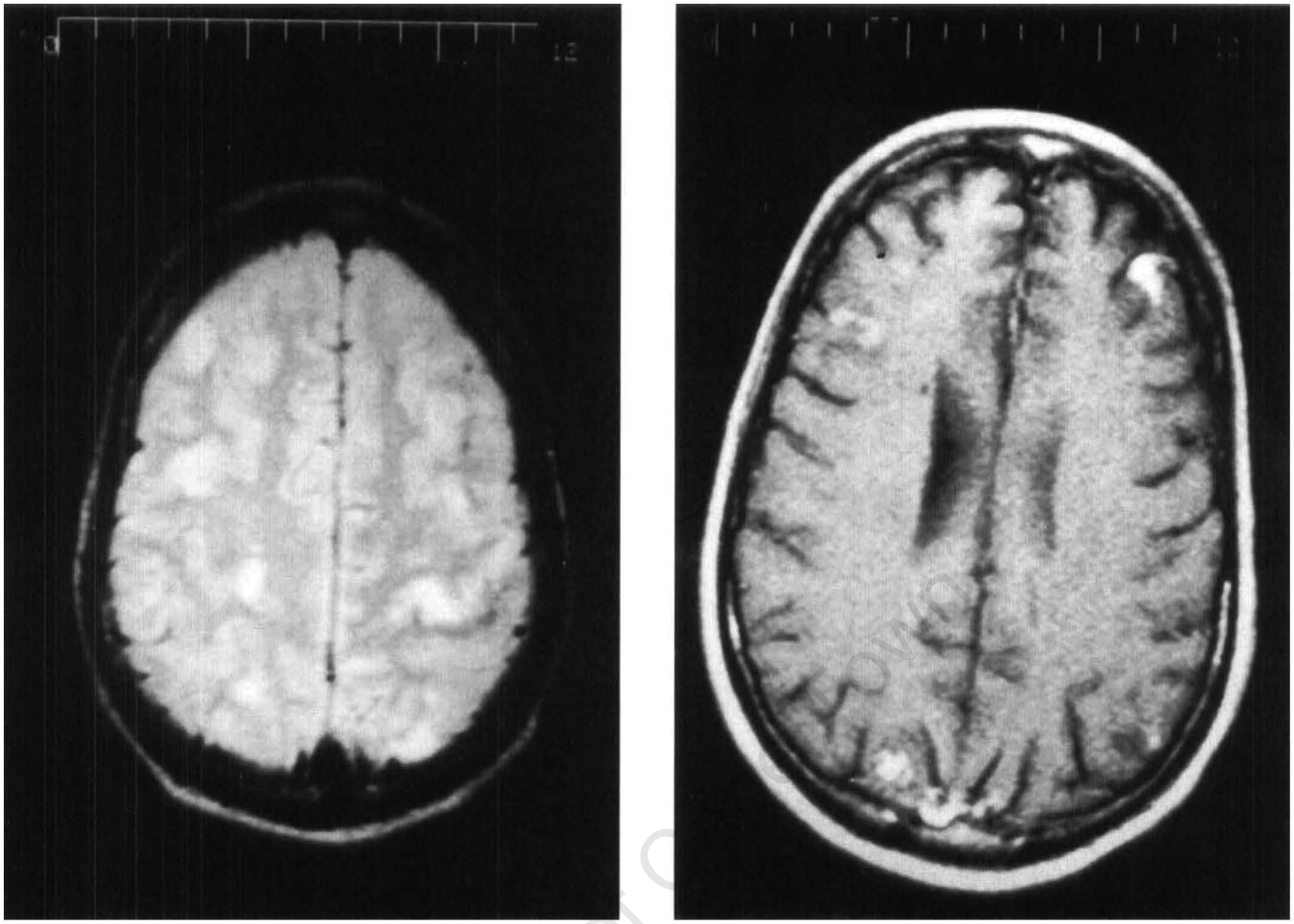
Patient 3. A 63-year-old woman with renal cell carcinoma of the sternum tolerated an initial course of IL2 followed after 1 week by PEG-IL2. A second, higher dose of PEG-IL2 was given 1 week later. Forty-eight hours after the second PEG-IL2 administration, she became confused, agitated, incontinent, and progressively somnolent. Her speech was dysarthric. Strength, tone, and deep tendon reflexes were decreased in the left arm and leg. Snout, root, and bilateral grasp reflexes were present. The left toe was upgoing. CSF analysis was normal. An MRI of the head showed possible bilateral occipital hyperintensities that had not been present on a baseline scan before admission.

The patient deteriorated. By the next day she was obtunded and unable to follow any commands and had intermittent decorticate posturing of her right arm. A repeat MRI showed multifocal white matter and cortical hyperintensities including definite bilateral occipital lesions (figure 2A). An EEG showed generalized slowing. Because the first two patients had seizures in a similar clinical setting, this patient was given phenytoin prophylactically.

At the time of neurologic deterioration, she had mild respiratory insufficiency, hypotension, and oliguria. Her WBC count was 29,000/mm^3 with 50% eosinophils. Her serum CK rose to 694. The prothrombin time was slightly prolonged at 18.6; other coagulation studies were normal.

The patient continued to worsen. By the fifth day after the last IL2 infusion, she could open her eyes spontaneously but did not focus visually, follow objects, or respond consistently to visual threat. Her pupils were equal and reactive. She appeared unaware of her surroundings and did not follow commands. There was no spontaneous movements of her arms or legs, and the left side did not withdraw to pain.

Over the next several weeks, the left spastic hemiparesis persisted. Noxious stimuli to the right side of the body elicited flexor posturing. Myoclonic jerks were intermittently present in the right arm. A CT without contrast



A B

Figure 1. (A) T_2-weighted MRI of patient 2 showing multiple cortical lesions. The lesions did not enhance with gadolinium. (B) T_1-weighted MRI with gadolinium obtained 1 week after that in A showing new enhancement of lesions.

showed multiple well-circumscribed lucencies throughout the cerebral hemispheres (figure 2B).

There was only slight improvement over the second month of hospitalization. Although she became responsive to her surroundings and was able to speak, she had irritability and marked deficits in attention and concentration. She was able to recognize her family and some objects. All limbs were spastic and hyperreflexic, and she had no movement of her right hand. Bilateral grasps persisted. She was transferred to a rehabilitation facility 7 weeks after receiving her final dose of IL2.

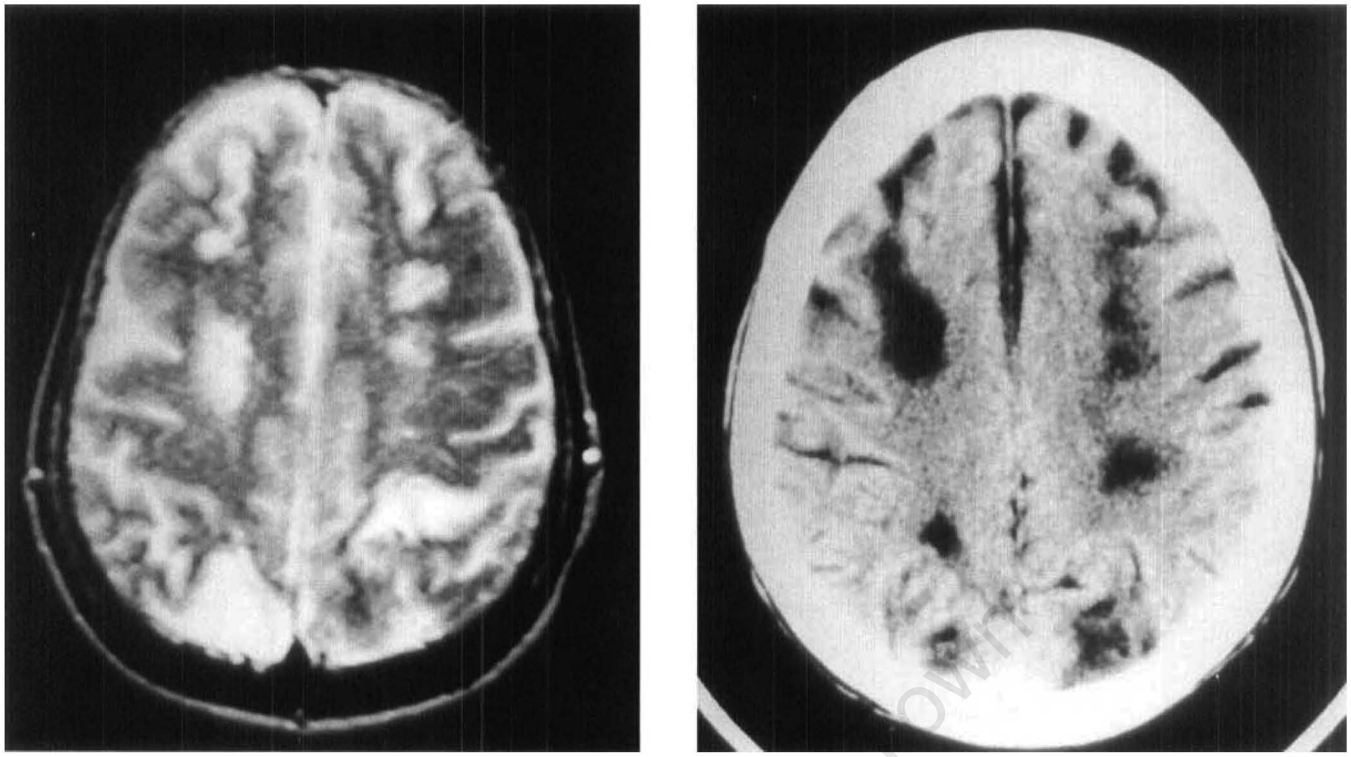
Patient 4. A 43-year-old HIV-seropositive man on azidothymidine began weekly doses of PEG-IL2. Approximately 60 hours after the sixth infusion, he became confused and unsteady. The following day he complained of mild bifrontal headache and was unable to feed or clothe himself properly. On examination, he was alert and oriented to person and place, but not date. His answers were slow, and he was unable to follow multi-step commands or do even simple arithmetic. Left-right confusion and perseveration were present. He could neither write clearly nor copy figures.

Cranial nerve examination was normal as was sensation to pinprick, light touch, vibration, and position. He had agraphesthesia and astereognosis. Strength was normal. Rapid alternating movements were performed poorly, and he was unable to walk with a tandem gait. Babinski responses were present bilaterally.

Laboratory studies included a WBC count of 18,700/mm^3 with 41% eosinophils. CD4 count was 450 cells/μL. LDH was elevated at 2,286 IU/L, and AST at 139 IU/L. Renal function was normal. The CSF contained nine RBCs/mm^3 and five WBCs/mm^3. The CSF protein was 57 mg/dL.

Two CTs were unrevealing. MRI 3 days after admission showed diffuse, nonenhancing white and gray matter lesions. An EEG showed left temporal slowing with bursts of frontal intermittent rhythmic delta activity. The patient improved rapidly and was neurologically normal at discharge on the fifth hospital day.

Patient 5. A 28-year-old woman with metastatic malignant melanoma received IL2 after failing to respond to conventional chemotherapy. After her seventh dose of IL2, she developed renal and respiratory insufficiency, hypotension, and peripheral edema. She required a brief period of respirator support. After extubation, the patient was confused. Although awake and alert, she was oriented to person only. She had little spontaneous speech, mild word-finding difficulty, poor repetition of words, and



A B

Figure 2. (A) T_2-weighted MRI of patient 3 showing multiple cortical and subcortical lesions. (B) CT obtained 3 weeks after A. The lesions appear as multiple cortical and subcortical lucencies.

perseveration. The deep tendon reflexes were brisk, and plantar responses were extensor. A head CT was normal. However, MRI showed a hyperintense lesion in the left parietal lobe. The CSF was normal. The patient's symptoms cleared completely over 5 days. The lesion was smaller but still visible on scans performed up to 10 months later.

Patient 6. A 56-year-old man with metastatic renal cell carcinoma was referred for immunotherapy after a radical nephrectomy. He completed the first course of eight doses of IL2 without difficulty. One week later, he was lethargic and had recurrent episodes of vertigo lasting about 15 minutes. He complained of unsteadiness while walking and a loss of grip strength in the left hand. The symptoms cleared for 2 days, then recurred along with blurred vision. A left homonymous hemianopia and bilateral limb ataxia were found on examination.

At the time the neurologic symptoms developed, renal function was normal, but the alkaline phosphatase and AST were slightly increased. The peripheral WBC count was 13,000/mm^3 with 2% eosinophils. The CSF contained 3 WBC/mm^3 and was otherwise normal. A head CT was normal, but an MRI showed multiple hyperintense lesions on the T_2-weighted scan. Lesions involved both gray and white matter and were found in the occipital cortex, left thalamus, and cerebellar hemispheres. They did not enhance.

The patient improved over 8 days. MRI 1 week after the initial scan showed the earlier lesions, but additionally found that the right occipital lesion enhanced with gadolinium. All lesions were smaller on a scan obtained 6 weeks later.

Patient 7. A 48-year-old man with metastatic renal cell carcinoma became confused and lethargic after two infusions of IL2 and GM-CSF. His neurologic examination was otherwise normal, as was MRI. Further immunotherapy was stopped because his white blood cell count had risen to 95,700/mm^3 with 55% eosinophils. Hydrocortisone and hydroxyurea were given to decrease the white blood cell count. At the time neurologic symptoms began, his renal function was normal; LDH and alkaline phosphatase were elevated to twice normal levels.

One day later, the patient deteriorated. He complained of dizziness and a headache and was disoriented with cognitive difficulties, hallucinations, expressive aphasia, and right-hand weakness. An EEG showed generalized and right frontal slowing. The CSF was normal. A second MRI showed nonenhancing, white matter hyperintensities in both frontal lobes, occipital poles, right parietal lobe, and right parietotemporal junction. MR angiography was normal. A third MRI 1 day later showed the previous white matter lesions and new signal abnormalities in the gray matter of the medial frontal lobes bilaterally. The patient gradually improved. By 6 weeks after admission he noticed only a slight impairment of fine motor movements when playing piano.

Patient 8. A 52-year-old man who underwent nephrectomy for renal cell carcinoma in 1986 had a recurrence of disease in 1994. He received IL2 and GM-CSF for 4 days. The second week of therapy was interrupted by severe diarrhea. The doses of both drugs were halved when treatment was restarted, and that of GM-CSF was reduced further when the absolute eosinophil count reached 20,000. During the third week of treatment, the patient

Table *Patient demographics and drug doses*

Patient	Sex/Age	Diagnosis	Drug/Dose*	Time of Symptom Onset
1	M/53	RCC	H-L IL2 4.5 mU/m^2 × 11 doses alpha-IFN 3 mU × 11 doses	22 days after start of treatment
2	M/41	RCC	First course:	3 days after start of second PEG-IL2 course
			IL2: 720,000 IU/kg × 7 doses	
			PEG-IL2: 3 mU/m^2 × 1 dose	
			PEG-IL2: 6 mU/m^2 × 3 doses	
			Second course:	
			IL2: 720,000 IU/kg × 7 doses	
			PEG-IL2: 3 mU/m^2 × 1 dose	
			PEG-IL2: 6 mU/m^2 × 1 dose	
3	F/63	RCC	IL2: 720,000 IU/kg × 7 doses	3 days after second PEG-IL2 dose
			PEG-IL2: 3 mU/m^2 × 1 dose	
			PEG-IL2: 6 mU/m^2 × 1 dose	
4	M/43	HIV	PEG-IL2: 3 mU/m^2 × 6 doses	3 days after sixth PEG-IL2 dose
5	F/28	Melanoma	IL2: 720,000 IU/kg × 7 doses	6 days after seventh IL2 dose
6	M/56	RCC	IL2: 720,000 IU/kg × 8 doses	8 days after first course IL2 completed
7	M/48	RCC	IL2: 1.5 mU/m^2 × 8 doses	6 days after eighth IL2 dose
			GM-CSF: 5 μg/m^2	
8	M/53	RCC	IL2: 1.5 mU/m^2 × 6 doses	2 days into second week of treatment
			GM-CSF: 5 μg/m^2	

* H-L IL2: Hoffman-LaRoche; other patients received the Chiron/Cetus preparation.

RCC = renal cell carcinoma.

became confused and restless. He was able to follow only simple commands and had poor attention with perseveration. A right homonymous hemianopia was present as well as right-sided neglect and ocular apraxia. There was right facial and arm weakness, hyperreflexia, and bilateral Babinski signs.

At the time of neurologic deterioration, the white blood cell count was 41,000/mm^3 with 50% eosinophils. The platelet count was 505,000/μL, and serum viscosity was increased to 4.3 (normal, 1.4 to 1.8). Serum protein electrophoresis was normal. Fibrinogen level was normal and fibrin split products were absent. CSF was normal. CT and MRI scans showed nonenhancing lesions in the left cerebellum and left frontal deep white matter. Repeat MRI several days later showed new nonenhancing lesions in both gray and white matter involving the thalamus, cerebellum, and occipital lobes. A third MRI the next day showed gadolinium enhancement in the left cerebellar, occipital, and posterior frontal lesions.

The patient gradually improved. Six weeks later, examination showed slight right arm weakness but was otherwise normal.

Results. The patients included six men and two women ranging in age from 28 to 63 years (mean age, 48 years). Six patients received IL2 for renal cell carcinoma, one for melanoma, and one for HIV infection (table). IL2 was the sole immunotherapeutic agent in five patients, three of whom received the long-acting preparation, PEG-IL2. One patient received alpha-IFN and two patients received GM-CSF in addition to IL2.

Neurologic symptoms did not begin immediately with the start of IL2 treatment. Five patients became symptomatic 3 to 8 days after completing a course of therapy, the other three during a course of treatment.

In each patient, the first sign of deterioration was a change in mental status with confusion, disorientation, and agitation. Three patients had hallucinations. Two patients became obtunded and one comatose. Two patients had seizures. Speech difficulties, including aphasia, dysarthria, and mutism, were present in four patients. Two patients had cortical blindness, and two had a homonymous hemianopia. Corticospinal tract involvement was manifest in seven of the eight patients who had weakness, spasticity, hyperreflexia, or Babinski signs. Three patients had perseveration, and one had primitive reflexes, including snout, bilateral grasp, and rooting reflexes. Four patients had limb or gait ataxia. Two patients had hemisensory deficits. One patient had left-right confusion, agraphesthesia, and astereognosis.

All patients had abnormal brain imaging. Multiple lesions were present in both gray and white matter in cortical and subcortical territories except for one patient who had an isolated left cortical parietal lesion. The lesions were not apparent or hypodense on CT (see figure 2B). On MRI, the lesions were either not visible or hypointense on T_1-weighted images, and hyperintense on T_2-weighted images. In one patient, the lesion enhanced with gadolinium on the first MRI scan obtained. In three other patients, lesions that did not enhance on the initial MRI scans did show enhancement on scans obtained 5 to 7 days later (see

figure 1). MR angiography, performed in only one patient, was normal.

Seventy-five percent of the patients had occipital lobe involvement, which was often bilateral. Half had parietal lesions and half frontal lesions. The cerebellum was involved in 33% of patients and the thalamus in 25%. No patient had brainstem lesions.

EEGs, recorded in five patients, showed generalized slowing. Additional focal slowing was noted in two patients. One patient had periodic localizing epileptiform discharges, and one patient had frontal intermittent rhythmic delta activity. CSF analysis was usually normal. Two patients had a slight increase in CSF protein. Three patients had a mild lymphocytic pleocytosis.

At the time of neurologic compromise, one patient had renal failure and several others had mild renal or hepatic dysfunction. Two patients had hypotension, which improved with intravenous fluid infusion. Routine coagulation studies were usually normal. The serum viscosity was elevated in the single patient in whom it was measured. All patients except one had an IL2-induced eosinophilia. Eosinophilia was mild in one patient whose absolute eosinophil count was 700/mm^3, but was marked in the others whose absolute eosinophil counts ranged from 1,372/mm^3 to 57,000/mm^3.

No patient received additional IL2 after the development of neurologic symptoms. While patients did not receive specific treatment for their neurologic symptoms, all received supportive care. Several required medication for agitation or seizures. Seven patients had complete or nearly complete recovery of neurologic function. Improvement usually began within a week of the onset of neurologic symptoms. One patient, however, had only minimal recovery. This patient remained severely encephalopathic with a spastic quadriparesis.

Discussion. The patients presented here developed neurologic illness during or shortly after treatment with IL2 with corresponding cortical and subcortical lesions on imaging studies. The initial behavioral changes were often similar to the more common IL2-induced encephalopathy, but focal signs rapidly evolved reflecting the areas of the brain affected.

There was no evidence to suggest an infectious or malignant cause for the neurologic illness. Patients with nervous system metastases were excluded from the IL2 trials, and CSF cytology and cultures were negative. Metabolic disturbances, including hyponatremia, hepatic failure, renal insufficiency, or hypoxia, were present in some of our patients, but they are not likely to have caused the neurologic syndrome as they were often mild and transient. One patient had renal failure severe enough to require dialysis, but the others had only oliguria or a slight increase in BUN and creatinine. The disturbance in three patients was limited to transaminase elevation. No single abnormality was uniformly present in all patients, and none is known to cause focal symptoms with multiple cortical and subcortical lesions.

Two of the patients were co-treated with GM-CSF and one with alpha-IFN. The other five patients received IL2 alone. Three of the patients received IL2 conjugated to polyethylene glycol (PEG), which decreases the clearance rate 15-fold.[18] The neurologic illness, however, cannot be due to GM-CSF, alpha-IFN, or the PEG moiety, since nonconjugated IL2 alone caused a similar picture and GM-CSF has no known neurologic complications. This neurologic toxicity occurred in 2 of 92 patients treated with PEG-IL2 at the National Cancer Institute. While PEG-IL2 may prolong exposure to high levels of IL2, in the absence of a prospective study it is not clear that neurologic complications are more frequent in patients receiving PEG-IL2 than in those receiving unconjugated IL2.

Patients with hypereosinophilia may develop peripheral neuropathy, cerebral infarction, or dementia with white matter lesions on MRI.[19-22] While we cannot dismiss the possibility that hypereosinophilia contributed to the development of neurologic symptoms, marked eosinophilia was not present in all of our patients, and there are no previous reports of this constellation of signs and reversible lesions in patients with idiopathic hypereosinophilia.

There are a few reports of similar neurologic illness associated with IL2. Aphasia, hemiparesis, seizures, visual distortions, and ataxia occurred in a few patients receiving IL2[12] or PEG-IL2[6] for metastatic renal cell carcinoma reported by Philip et al. and Bukowski et al. Meyers and Yung[23] described a melanoma patient who developed a cerebellar syndrome and dementia 3 months after completing treatment with IL2 who had multiple white matter lesions on a later MRI scan.[23] The melanoma patient of Somers et al.[15] developed a spastic quadriparesis following IL2 with subcortical and cortical hyperintensities on MRI. The neurologic symptoms and the lesions gradually resolved. Bernard et al.[11] described two IL2 patients with brief episodes of focal neurologic deficits. Brain imaging was normal in both. Vecht et al.[16] reported a melanoma patient who developed a rapidly fatal encephalopathy with blindness, ataxia, and bilateral Babinski signs during an initial course of IL2. A CT was normal; MRI was not reported. At autopsy, the gray matter was normal but the white matter contained multiple small foci of perivascular demyelination. Because of the pathologic resemblance to acute perivenous encephalomyelitis, they postulated that the lesions were autoimmunologically mediated.

IL2 penetrates the blood-brain barrier. The CSF IL2 concentration averages 50% of serum level.[24] Specific cerebral IL2 binding sites are present in rat brain with highest density in the hippocampus. Similar receptors are likely present in human brain.[25,26] IL2 can alter adrenergic, dopaminergic, and cholinergic neurotransmission.[25,27-30] Direct neuromodulatory effects mediated by endogenous IL2 receptors may underlie the acute delirium frequently associated with IL2 therapy,[28,29] but are unlikely to have caused structural lesions.

IL2 can both promote the proliferation and matu-

ration of oligodendrocytes[31] and damage myelin.[32,33] Demyelination was the primary pathology in the case of fatal IL2 encephalopathy reported by Vecht et al.[16] There was, however, no clear evidence of myelin breakdown in our patients. The lesions affected both gray and white matter, and the CSF protein, which is often elevated in demyelinating diseases, was normal in six of the eight patients.

IL2 causes a systemic capillary leak syndrome.[34] Although Elison et al.[32] reported increased cerebral vascular permeability in cats, others have not found blood-brain barrier disruption with IL2 infusion.[35,36] The absence of gadolinium enhancement at the time of disease onset in our patients argues against blood-brain barrier dysfunction as a precipitating factor in the illness.

The brain lesions in our patients were not typical of arterial strokes. Multiple arterial territories were affected simultaneously and lesions resolved quickly. The subacute evolution of lesions, absence of hemorrhage, and lack of an embolic source make it unlikely that the lesions were caused by emboli.

IL2 induces IL1 and tumor necrosis factor (TNF) which affect endothelial cell function and coagulation.[2,34,37,38] TNF alone can damage axons and myelin in vitro and can cause a transient aphasia and focal neurologic deficits in patients.[10,37,39] IL2 may, therefore, cause neurologic dysfunction by inducing other cytokines.

IL2 can prolong the prothrombin time (PT) and partial thromboplastin time (PTT) and decrease coagulation factors,[40,41] but the PT and PTT were usually normal in our patients. More extensive monitoring of coagulation at one of our institutions showed an increase in von Willebrand's factor, but no abnormalities in protein C, protein S, antithrombin III, fibrinogen, PT, or PTT in patients receiving IL2.

The pattern of illness and distribution of lesions associated with IL2 are similar to those complicating cyclosporine therapy and occasionally seen with chemotherapy, alpha-IFN, hypertensive encephalopathy, and eclampsia.[42-53] The etiology of neurologic disease in these circumstances is unknown, but has been attributed to direct toxic effects of medications, cortical venous thrombosis, or a local vasculopathy with fluid extravasation. Cytokine activation is also likely in all these circumstances.

In summary, we describe eight patients who developed cortical and subcortical neurologic signs and cerebral lesions during treatment with IL2-based immunotherapy. The etiology of this illness is not known, but the similarity to neurologic complications of cyclosporine, chemotherapy, and other immunotherapy suggests a common pathogenesis.

The occurrence of new neurologic signs and lesions in patients receiving IL2 should not be presumed to be progression of the underlying disease. Since the neurologic illness associated with IL2 may be completely reversible, further IL2 infusion should be stopped and supportive care provided to maximize neurologic recovery.

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Is HIV/AIDS a primary-care disease? Appropriate levels of outpatient care for patients with HIV/AIDS

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Objective: To estimate the proportion of outpatient visits that could be managed at a primary-care level, by World Health Organization (WHO) clinical staging.

Design: Prospective, descriptive study. Six medical doctors in a tertiary hospital HIV ambulatory clinic recorded clinical diagnoses, WHO clinical staging and their recommendation regarding the appropriate level of care for each outpatient seen.

Setting and study population: All HIV-infected patients attending a public-sector, urban, South African, referral and teaching hospital HIV outpatient clinic between September and November 1992.

Participants: There were 238 visits by 148 patients during the study period.

Results: Of 238 visits, 165 (69.3%) were deemed suitable for treatment at the primary-care level. After allowing for contradictory responses, at least 141 visits (59.2%) could be appropriately treated at the primary-care level. Although all six doctors assessed more than half of their visits as suitable for primary care, there were significant differences among them. In total, 83 visits (34.8%) needed a medical specialist, and 45 (18.9%) required tertiary-care facilities. Of all the visits, 58 (24.9%), 51 (21.9%), 60 (25.8%) and 64 (27.4%) were classified as WHO stages 1, 2, 3 and 4, respectively. For these stages, 55 (94.8%), 38 (74.5%), 42 (70.0%) and 26 (40.5%) visits, respectively, were suitable for treatment at a primary-care facility.

Conclusions: Many of the outpatient visits to this outpatient specialist clinic could have been safely cared for at a primary-care level. As the severity of the disease increases, there is a decrease in the proportion of patients that can be treated at a primary-care level.

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Keywords: AIDS, primary care, ambulatory care, referral, appropriateness, severity, staging

Introduction

As the number of HIV-infected individuals in South Africa continues to rise, referral hospitals, which are presently the major site at which treatment occurs, will accumulate more HIV-infected patients [1]. Recent research estimates that by 2005 up to 75% of the South African health-care budget could be consumed by HIV-infected individuals if the current approach to HIV treatment is maintained [2]. However, a number of studies in South Africa have shown that referral hospitals provide unnecessarily and ineffectively sophisticated care for a large proportion of their in- and outpatients, many of whom could have been safely treated in primary-care facilities. One study concluded that 30% of all inpatient days in referral hospital internal medicine beds could

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have been appropriately managed in a less sophisticated facility [3].

This study gathers clinician's opinions on the level of health care appropriate to the needs of their HIV-infected outpatients attending the Somerset Hospital. At the time of the study, this was the only centre providing HIV care in the city of Cape Town (population, 2 million [4]).

This study limits its focus to clinical conditions, rather than to patient's feelings, or their social and personal circumstances. Counselling and other social and psychological support systems have long been community oriented, and are, in our setting, more often based in communities or in primary-care clinics than are specialist clinical services. The reluctance to decentralize clinical functions has been the main obstacle to basing more HIV care in the community and in primary-care facilities.

The purpose of this study is to contribute to the integration of care for HIV disease within the overall restructuring of health services towards the primary health-care approach [5,6], and to contribute to the planning of health care for the HIV epidemic.

Our objectives were (1) to determine the proportion of outpatient visits that could have been safely cared for at a primary-care level, according to the clinical staging described by the World Health Organization (WHO), and (2) to describe outpatient visits in terms of the elements of clinical care provided: diagnosis, tests, and specialist services.

Methods

The study population consisted of all HIV-infected patients attending the city's only HIV clinic, the Somerset teaching and referral hospital, between September and November 1992. At the time of the study all public-sector patients and many of those with private medical insurance were referred there on diagnosis. The HIV clinic operates 1 day each week. At their first visit, patients from a single queue are sequentially allocated to doctors as they become available, in an essentially random manner. Thereafter they remain under the care of that doctor.

For every consultation, the six medical doctors in the clinic (two internal medicine specialists and three general practitioners with specific HIV expertise and one general practitioner new to HIV clinical management) recorded information on clinical diagnosis, WHO clinical HIV stage [7], management, follow-up and appropriate level of care.

Three complementary questions (Table 1) were answered as to level of care. The first question asks the clinician to judge whether the patient could have received appropriate care for that visit if it had taken place at the primary-care level, with a suitably trained primary-care practitioner.

Table 1. Level of care by practitioner.

	n (%)	
Observer	'Yes' or 'probably'	Total
Patient visits that could have been appropriately managed at a primary-care centre by a suitably trained primary health-care practitioner:		
1	26 (56.5)	46 (19.3)
2	27 (65.8)	41 (17.2)
3	50 (76.9)	65 (27.3)
4	10 (52.6)	19 (7.9)
5	44 (80.0)	55 (23.1)
6	8 (66.6)	12 (5.1)
Total	165 (69.4)	238 (100)
95% CI	(63–75)	
Patient visits that needed specialist skills or knowledge:		
1	19 (41.3)	46 (19.3)
2	14 (34.1)	41 (17.2)
3	34 (52.3)	65 (27.3)
4	6 (31.5)	19 (7.9)
5	9 (16.4)	55 (23.1)
6	1 (8.3)	12 (5.1)
Total	83 (34.8)	238 (100)
95% CI	(29–41)	
Patient visits that required tertiary-care facilities:		
1	9 (19.5)	46 (19.3)
2	12 (29.2)	41 (17.2)
3	6 (9.2)	65 (27.3)
4	8 (42.1)	19 (7.9)
5	9 (16.3)	55 (23.1)
6	1 (8.3)	12 (5.1)
Total	45 (18.9)	238 (100)
95% CI	(14–24)	

CI, confidence interval.

'Appropriate' care was defined as care that is as good as the care provided by the tertiary hospital clinician for the patient at that visit. The primary-care level in South Africa would be a small clinic, not attached to a hospital, and with no laboratory or radiology facilities on site. A limited drug list would, for example, include cotrimoxazole and non-systemic antifungal agents, but would exclude zidovudine. It would be staffed by nurses, with training in diagnosis and treatment of common conditions, with regular visits from a general practitioner. A larger health centre may have a full-time medical general practitioner on the staff, a wider drug list, and basic radiology and laboratory facilities.

A 'suitably trained' primary-care practitioner was defined as a clinical nurse, or a non-specialist medical doctor working in a primary-care facility who has undergone appropriate short training courses in HIV management. This was described as including 5 days of clinical teaching by infectious disease specialists at the HIV clinic

at Somerset Hospital, with a regular refresher course of similar length and method.

The second and third questions asked whether the clinician believed that the level of clinical skill and the technical facilities (diagnostic and therapeutic) required for the patient at that visit could only be provided in a tertiary setting.

These questions could be answered 'yes', 'probably', 'unlikely' or 'no'. Apart from a standard description of the definitions of levels of care, no attempt was made to ensure a consensus among the doctors on the appropriate level of care for different clinical conditions.

Statistical analysis

The data were coded, and analysed using SAS [8]. The χ^2 test or Fisher's exact test was used where appropriate and 95% confidence intervals were calculated.

Results

Over the study period, a total of 148 patients had 238 consultations.

Level of Care

Of all visits, 69.4% (range, 52–80%) were considered suitable for primary care (Table 1); 102 (42.9%) visits had a definite positive answer to this question, while 63 (26.5%) visits were rated as 'probably'. There were significant differences between practitioners (Fisher's exact test, 11.02; $P=0.047$).

Specific needs

One-third of all visits needed a medically trained specialist, and one-fifth required tertiary hospital facilities (Table 1). There were significant differences between the clinicians' opinions on these proportions ($P=0.001$ and $P=0.01$, respectively).

Although 165 (69.3%) visits were rated as suitable for primary care by the first question, 21 (12.7%) were deemed to need specialist care, nine (5.4%) to need tertiary facilities and three (1.8%) to need both. These contradictions amount to 20.0% of the visits suitable for primary care and 13.8 % of all visits. Of the 73 visits which were classified as not being suitable for the primary-care level, 31 (42.4%) needed both tertiary facilities and specialist skills, 28 (38.3%) needed specialist skills only, and five (6.8%) needed tertiary facilities only. There were nine (12.3%) contradictions (i.e., needed neither a specialist nor tertiary facilities). Taking these contradictory responses into account, at least 141 (59.2%) visits could be appropriately treated at the primary-care level.

Stage of disease and level of care

As the severity of the disease increases, there is a significant decrease (Mantel–Haenszel χ^2 test for trend, 40.0; $P<0.0001$) in the proportion of patients that can be treated at a primary-care level (Table 2). Of the WHO stage 4 visits, 59.5% could not have been adequately managed at a primary health-care level ('definitely not', 36.5% and 'unlikely', 23%). If the 14 contradictory responses are included, then only 18.7% of stage 4 visits could have been cared for at a primary-care level.

Table 2. Level of care by World Health Organization (WHO) stage of patients.

	'Yes' or 'probably'		Total
WHO stage	n (%)	95% CI	n (%)
Patient visits that could have been appropriately managed at a primary-care centre by a suitably trained primary health-care practitioner:			
1	55 (94.8)	84–99	58 (24.9)
2	38 (74.5)	59–86	51 (21.9)
3	42 (70.0)	59–82	60 (25.8)
4 (AIDS)	26 (40.5)	28–53	64 (27.4)
Total	161 (69.1)	63–75	233* (100.0)
Patient visits that needed specialist skills or knowledge:			
1	5 (8.6)	3–18	58 (24.9)
2	16 (31.3)	18–44	51 (21.9)
3	18 (30.0)	18–42	60 (25.7)
4 (AIDS)	42 (65.6)	54–77	64 (27.5)
Total	81 (34.7)	29–41	233* (100.0)
Patient visits that required tertiary-care facilities:			
1	5 (8.6)	3–18	58 (24.89)
2	10 (19.6)	9–32	51 (21.89)
3	8 (13.3)	6–24	60 (25.75)
4 (AIDS)	22 (34.4)	23–46	64 (27.47)
Total	45 (19.3)	14–24	233* (100.00)

*Total is less than 238 because five visits were not staged. CI, confidence interval.

As the severity of the disease increases, there are significant increases in the proportions of patients who needed to be seen by a specialist ($\chi^2=40$; $P<0.0001$), and who required tertiary-care facilities ($\chi^2=40$; $P=0.001$) (Table 2).

Diagnosis

There were 349 diagnoses: an average of 1.4 diagnoses per visit. Mucocutaneous disorders were the most common problems encountered (Table 3), most of which (75.5%) could be cared for at a primary-care level.

Of the 14 visits with a diagnosis of oral candidiasis which were rated as not suitable for primary health care, 10 had other diagnoses [lymphoma, pancytopaenia, Kaposi's sarcoma (two), atypical mycobacteriosis, wasting syndrome, peripheral neuropathy, oesophageal candidiasis, pulmonary tuberculosis and anaemia].

The total number of investigations requested during the period of study was 214, or 0.9 tests per visit. More than half of all visits (128; 53.9%) had no laboratory tests ordered. Patients deemed appropriate for treatment at a primary-care level needed 0.65 tests per visit, most commonly a full blood count with CD4 lymphocyte

Table 3. Common diagnoses*.

Diagnosis	No. (%) suitable for primary care	95% CI	Total
Mucocutaneous disorders†	34 (75.5)	59–87	45
Asymptomatic follow-up	25 (86.1)	68–96	29
Oral candidiasis	10 (41.6)	22–61	24
Pulmonary tuberculosis	11 (64.6)	42–87	17
Kaposi's sarcoma	8 (50.0)	26–75	16
Persistent glandular lymphadenopathy	9 (81.8)	48–98	11
Urinary tract infection	6 (85.7)	45–99	7
Unexplained cough	2 (28.6)	4–68	7
Total	105 (67.3)	6–75	156

*Other diagnoses each <2% prevalence. †Sebhorraeic dermatitis, prurigo, fungal skin and nail infections, recurrent oral ulcerations, angular cheillitis, Herpes Zoster, scabies, molluscum contagiosum. CI, confidence intervals.

count and a chest radiograph. Common microbiology tests included syphilis serology, hepatitis B tests and pus and sputum microscopy and culture.

Follow-up

Routine follow-up of an asymptomatic patient constituted a relatively high percentage of all consultations (12%) and diagnoses (8.3%). Over 85% of these could be moved to primary care.

Discussion

All doctors independently assessed that more than half of their patients' visits could be managed by trained clinicians at a primary-care level. Further studies are needed in other centres to confirm the generalizability of this finding.

The differences between practitioners for the first question (on the level of care) and the contradictory answers are not large enough to change the conclusion of the study. Variation between otherwise similar physicians in decision making and consequent clinical practice is well known [9].

Instead of using subjective opinions, objective criteria could have been developed to assign patients to a specified level of care [3]. However, the complexity of deciding on an appropriate level of care for a given patient will always entail subjective judgment [10], and given that the regular clinician makes frequent decisions about patients' therapy, he or she is ideally positioned to assess the appropriate level of care for their patient.

In contrast to other studies (H. Schneider, personal communication, 1993), which used retrospective record reviews to assess the level of care needed for adult medical outpatients, data for this study were collected prospectively at the time of consultation. This ensured that information was fresh in the minds of the practitioners.

The WHO staging clinical system for HIV disease [7] proved useful in distinguishing subgroups of patients more likely to need tertiary care. The higher percentage of stage 4 (AIDS) patients that required a specialist compared with the percentage that required tertiary facilities (Table 2) may indicate that it is the skill and experience of the specialist rather than sophisticated diagnostic or therapeutic facilities which are needed in the late stages of the disease.

Although diagnosis of skin conditions in the early stages requires skill and experience, the study results suggest that a suitably trained clinician could have managed these problems adequately, using a limited range of medications.

Chronic unexplained cough, oral candidiasis and Kaposi's sarcoma were associated with a higher percentage of visits, which could not be adequately treated at a primary-care level. An unexplained cough often requires radiological and laboratory support for diagnostic work-up and exclusion of treatable opportunistic infections like *Pneumocystis carinii* pneumonia (PCP) and tuberculosis.

Pulmonary tuberculosis often, but not always, requires tertiary levels of expertise for diagnosis, early treatment and stabilization, but could be treated at a primary-care level thereafter. Oral candidiasis, provided it is not accompanied by other more severe and complicated diagnoses, is usually easy to diagnose and treat at lower levels of care. Kaposi's sarcoma requires specialist referral, for diagnosis and care.

South African public-sector referral and teaching hospitals are already having to cope with restricted funding and an increasing patient population, especially in urban areas [11]. The emergence of HIV infection will overwhelm health care as it is currently organized.

This study has highlighted the urgent need for HIV infection to be seen in the context of the primary health-care approach. The results of this study suggest that all stage 1 visits, routine follow-up visits in other stages and selected symptomatic visits should be dealt with at the primary-care level. Clinics could be staffed by appropriately trained nurses, with larger health centres run by nurse and doctor teams, provided the clinicians receive appropriate training in managing HIV-related illness using clinical guidelines, and receive specialist support. These facilities need not have laboratories or radiological facilities. Specialist centres could then be more appropriately used caring for referred patients with complicated illness, developing the clinical guidelines and providing training, telephone, visit, and referral support to peripheral clinics and health centres.

Pilot programmes and evaluations are needed to assess the impact of decentralization on cost and quality of care, including patient perceptions. This would assist in decisions on policy and wider implementation of this system, which, in high prevalence areas, may well offer HIV-infected individuals efficient, humane and appropriate treatment.

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HIV infection: transmission hazards in life and death

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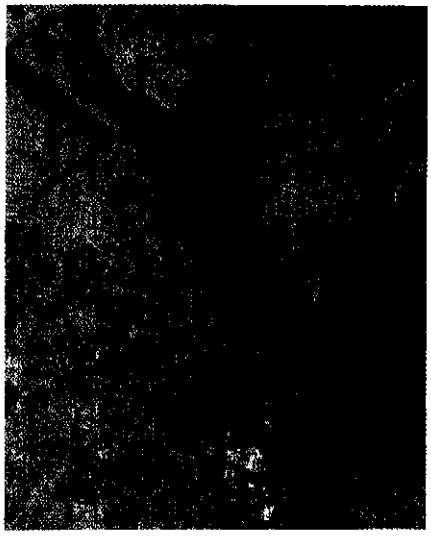
Inconsistencies in the application of universal precautions could lead to unnecessary exposure to and infection by HIV.

Increasing numbers of South Africans are becoming infected with the human immunodeficiency virus (HIV). The national HIV seroprevalence rate in women attending antenatal clinics in November 1994 was 7.5% and an estimated 1 in 40 of the general population may be infected. The epidemic is rapidly approaching the scale seen in other southern African countries, where HIV infection is the major cause of mortality in young adults and where more than 50% of hospitalised patients have HIV-related diseases. As the epidemic develops, significant numbers of patients will die as a result of HIV infection. This poses further hazards. Undertakers, embalmers, nursing staff, mortuary attendants and pathologists are increasingly called upon to handle HIV-infected cadavers. Undertakers and nurses perform procedures such as 'laying out a body', which are associated with minimal exposure to virus-infected tissues and body fluids. Anatomical pathologists and mortuary attendants perform highly invasive procedures such as autopsies, laparotomies, skin suturing, bone cutting and drilling with mechanical devices. As part of the process of body preservation, embalmers may be required to remove abdominal and cranial contents and inject preservatives into the vasculature under pressure.

There is a potential for transmission of HIV infection associated with each of these procedures and appropriate infection control measures should be utilised. The aim of this article is to review some of the available scientific data in order to allow a rational assessment of transmission risk from living and dead patients.

Current control policies

Fear of acquiring HIV infection, as well as the social stigma of AIDS, has led to the shunning of many HIV-infected individuals by health care workers (HCWs) and the general public. These



fears have not been limited to the living, but have led to undignified and extreme precautions for handling the dead. The belief that HIV-positive patients are easily identifiable has resulted in extraordinary precautions being taken with known HIV-positive individuals, while precautions for the unrecognised cases are ignored. Until 1994, the 'laying out' procedure for patients who died in Cape provincial hospitals included sealing known HIV-infected cadavers in double plastic bags and attaching 'Danger of Infection' labels. No added precautions were taken for cadavers of unknown HIV serological status.

Anatomical pathologists have also been reluctant to perform postmortem examinations on known HIV-infected patients. When postmortems are performed on these cadavers, they are limited in nature and special precautions are taken. However, no added precautions are taken when the HIV serological status is unknown. Patients known to be HIV-infected represent the 'tip of the iceberg' of the infected population. In the Western Cape, the region with the lowest HIV prevalence in South Africa, a prospective hospital study found that 33% of newly diagnosed tuberculosis patients were HIV seropositive. The present inconsistent application of infection control measures results in a false sense of security, while doing little to decrease the real risks. The epidemic is now well established in this country with significant numbers of all population groups infected.

Sampling and investigation of body fluids is an integral part of modern medicine and exposes HCWs to the risk of nosocomial HIV infection.

Assessment of HIV infection risk

Data on human transmission risk have been derived from *in vitro* virological studies and epidemiological observations. Virological studies have utilised HIV-1 culture techniques and very sensitive polymerase chain reaction technologies. These studies have demonstrated cell-associated virus, cell-free virus, viral RNA and pro-viral DNA in many human tissues and body fluids. They confirm that postmortem tissues and body fluids have the potential for HIV transmission. Epidemiological studies, in contrast, have generally demonstrated little transmission outside the conventional risk behaviours.

Community-acquired HIV infection

The main risk factors for acquiring HIV infection were recognised in the early stages of the AIDS pandemic and included sexual contact with an infected individual, parenteral exposure to infected blood and vertical transmission from an infected mother to her infant. The isolation of HIV from body fluids other than blood and semen raised concerns that 'casual' contact with patients might lead to infection. Subsequent long-term epidemiological studies of families and household contacts of AIDS patients showed no 'casual' transmission of HIV, despite many years of exposure.[1,2] They have established that the infection is extraordinarily difficult to transmit outside the recognised risk behaviours.[3]

Nosocomial transmission

Sampling and investigation of body fluids is an integral part of modern medicine and exposes HCWs to the risk of nosocomial HIV infection. Potential infection risks include: contact with blood on intact skin; contact with blood or body fluids on broken skin; contact of body fluids with the mucosa of eyes, mouth or nose; penetrating injuries caused by scalpel blades, needles, etc. and inhalation of aerosols from mechanical devices such as bone saws.

- Exposure of intact skin to infected blood does not appear to be a significant risk for acquiring HIV infection.[4]
- Non-parenteral exposure of non-intact skin to infected blood has been reported to result in infection on rare occasions.
- There is an estimated transmission risk of 1 in 2 500 following mucosal exposure.
- The most significant infection risk is associated with needle-stick injuries. The HIV seroconversion rate following hollow-needle penetration is approximately 1 in 250 exposures. Absolute risks are dependent on the quantity of blood transferred and the titre of virus in the HIV-infected blood. HIV is present in the blood in high titre at the time of seroconversion, declines rapidly and remains at low levels until finally rising with the onset of AIDS.
- Transmission by aerosol has not been documented.
- Exposure to body fluids other than blood poses a lower infectious risk as viral titres are usually lower than those found in blood.

Postmortem HIV viability

HIV has repeatedly been isolated from cadaveric blood and tissues,[5-7] although viral titres decline after death. Cell co-culture assays have shown that the tissue infectious titre of whole blood taken from HIV-infected patients decreased by approximately 1 log per day in 4

of 5 patients studied over a 4-day period.[8] Even though HIV may be present in a decreased titre in cadaver tissues, body fluids should be considered potentially infectious and refrigeration does not seem to decrease recovery of the virus. Epidemiological evidence of any postmortem HIV transmission is scanty. Despite the performance of many thousands of autopsies on HIV-positive cadavers, there have been no reports of pathologists seroconverting as a result of an injury acquired in a mortuary. Three mortuary technicians, however, have been reported to have acquired possible occupationally related HIV infection.

Recommended infection control measures

Control measures should be based on scientific principles, should not engender unnecessary fear and prejudice against HIV-infected patients and should not be seen in isolation from other common infectious diseases such as hepatitis B and tuberculosis. The HIV epidemic in South Africa now affects significant numbers of all age, sex and racial groups, necessitating the universal application of precautions. Infection control measures should be applied to everybody and not limited to those known to be HIV-positive. An alternative strategy would be to institute universal HIV testing before performing higher-risk procedures. The level of precaution should be related to the exposure to blood and tissues.

For minimal risk activities such as 'laying out' bodies disposable gloves and aprons should be worn, especially when blood contamination is present. Antiseptics should be used for neutralising contaminating body fluid leaks, and the body should be covered with a shroud. The transmission risk is low and probably comparable with that of household contacts of AIDS patients.

More invasive procedures such as

an autopsy have increased potential for HIV transmission. Besides the use of gloves, protective clothing and eye protection and suitable extraction venting of postmortem rooms, adequate skill and training are required. Protective measures should, however, not impede dexterity. Mechanical devices such as bone drills should cause minimal aerosol production.

Protection against inhalational exposures is more problematic. While there is no epidemiological evidence that this is a route of transmission, full protection would require a close-fitting face mask or plastic visor with down-draft air flow.

If professionals are called upon to perform procedures they perceive as risky, failure to address their concerns will prove counter-productive.

Added infection control precautions may add significantly to the cost of pathology services. It has been estimated that a postmortem on an HIV-positive cadaver in the UK cost R6 000, approximately 50% more than the cost of a conventional procedure.[5] If universal application is unaffordable, an alternative strategy would be the HIV testing of all cadavers which are subjected to postmortem examination.

Embalming is not a common procedure but has attendant transmission risks of at least similar magnitude to a postmortem. Infection control measures should be of a similar nature to those of a postmortem examination and should be applied universally. Such procedures, moreover, should only be performed by suitably trained individuals. In the USA and Europe, appropriate training of embalmers and mortuary attendants is ensured by a nationally recognised training programme, with subsequent registration. There is no such recognised training programme in South Africa.

Available epidemiological evidence indicates that postmortem transmission of HIV is very rare.[5] The risk of transmission is highest when the intact skin barrier is broached by a needle or scalpel. Infection control measures are applied inconsistently. Many of those at most risk have no recognised training and there are no professional bodies to ensure the maintenance of minimum standards.

There have been no systematic postmortem studies in South Africa and we are dependent on data from Zaire[9] and the Ivory Coast[10] to characterise AIDS manifestations in our communities. If professionals are called upon to perform procedures they perceive as risky, failure to address their concerns will prove counter-productive. The actual transmission risks in South Africa can only be assessed if ongoing surveillance data are collected throughout the country. Such data are required before individuals exposed to occupational infection can be reassured that the risk of transmission is insignificant. Nevertheless, the occupational risks appear to be low.

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IN A NUTSHELL

In the South African population 1 in 40 people are HIV-infected and the proportion is considerably higher in hospitalised patients.

The application of infection control measures only to known HIV-seropositive individuals gives a false

sense of security, while doing little to reduce HIV transmission.

Virological studies confirm that HIV remains viable in cadaver tissues and blood and these are potential sources of infection.

Epidemiological evidence indicates that exposure to HIV-infected blood is the major risk factor for nosocomial HIV transmission.

The risk for acquiring HIV infection from blood is approximately 1 in 2 500 for mucosal exposure and 1 in 250 for hollow needle stick injuries.

Rational HIV infection control measures should also be adequate for other common infectious diseases and they must be universally applied by suitably trained individuals.

IN 'N NEUTEDOP

In die Suid-Afrikaanse bevolking is 1 uit 40 mense met HIV geïnfekteer en die proporsie is aansienlik hoër in gehospitaliseerde pasiënte.

Die toepassing van infeksiebeheermaatreëls slegs vir bekende HIV-seropositiewe individue gee 'n vals gevoel van veiligheid terwyl dit min doen om die HIV-transmissie te verminder.

Virologiese studies bevestig dat HIV lewensvatbaar bly in kadawerweefsels en -bloed en dit is potensiële bronne van infeksie.

Epidemiologiese bewyse dui daarop dat blootstelling aan HIV-geïnfekteerde bloed die belangrikste risikofaktor vir nosokomiale HIV-transmissie is.

Die kanse om HIV-infeksie van bloed op te doen is omtrent 1 in 2 500 vir mukosale blootstelling en 1 in 250 vir holnaald prikbeserings.

Redelike infeksiebeheermaatreëls vir HIV behoort ook voldoende te wees vir ander algemene aansteeklike siektes en hulle moet universeel deur goed-opgeleide individue toegepas te word.

HIV TRANSMISSION

Quality of Life Research, 5, pp. 275–280

The impact of human immunodeficiency virus (HIV) infection on quality of life in a multiracial South African population

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We set out to document quality of life in South African HIV subjects, using the Medical Outcomes Survey (MOS) SF-36 instrument, and to determine whether this was affected by race, gender or clinical stage of disease. A cross-sectional survey of 134 HIV outpatients (42 White, 49 Mixed race, 43 Black) and 114 healthy non-medical hospital personnel (36 White, 37 Mixed race, 42 Black) was carried out at a referral centre for HIV patients in the Western Cape region of South Africa. Scores on eight scales measuring different aspects of quality of life were calculated. Black female controls scored significantly lower on all scales ($p<0.05$) except physical function. HIV-infected subjects of Mixed race (both genders) reported poorer physical function ($p<0.05$) but no other scale was affected by race. HIV subjects scored significantly lower than controls on all scales ($p<0.01$); the majority of the decline in function occurred early in disease by WHO stages 1 and 2. We conclude that HIV-infection impacts early on all aspects of quality of life and that this impact is largely independent of racial origin.

Key words: Human immunodeficiency virus (HIV); Medical Outcomes Survey (MOS) Instrument SF-36; race; quality of life; WHO Clinical disease stage.

Introduction

Random testing of patients attending antenatal clinics in South Africa in 1994 revealed an overall seroprevalence for Human Immunodeficiency Virus (HIV) infection of 8% (ranging from just over 1% in the Western Cape region to nearly 15% in Kwazulu/Natal) and is expected to rise to 20% by the year 2000.[1] The Cape Town population is comprised of Blacks (mainly of Xhosa origin), Whites (English and Afrikaans) and persons of Mixed race (English or Afrikaans speaking). Homosexually transmitted HIV-infection was first reported in Cape Town in 1982 in White and Mixed race males, but heterosexually acquired infection is now predominant and the majority of infected persons are Black or of Mixed race. Cape Town is thus ideally situated to study the potential effects of race on HIV disease presentation.

HIV-related quality of life is an important issue both for the infected individual and for society as a whole. Impairment of quality of life has been shown in Western AIDS patients. However, there is little data concerning quality of life at earlier stages of HIV-infection or race-related differences in quality of life[2,3,4,5] and no data for African HIV subjects. Given South Africa's political history, one might expect to find differences in quality of life between the population groups. However, there are problems associated with assessment of quality of life in Africa as comprehensive instruments are not available in the local languages and their content may not be appropriate in the different cultural setting. The Medical Outcomes Survey (MOS) instrument, MOS SF-36,[6] was selected for this study of quality of life in the Cape Town HIV population because it has been widely used internationally.[7] Translations into the other local languages, Afrikaans and Xhosa, had to be made and validated.

Methods

Setting

The HIV Clinic at Somerset Hospital, Cape Town is a well established referral centre for HIV-infected patients from the Western Cape region of South Africa. Whites, Blacks and people of Mixed race at all clinical stages of HIV disease are seen at the clinic.

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Patients are routinely counselled at the time of diagnosis. All HIV outpatient visits are recorded on a computer database which is updated weekly.

Instrument

The MOS 36-item Short-Form Health Survey (SF-36) assesses eight health concepts: (1) limitations in physical activities because of health problems; (2) limitations in social activities because of physical or emotional problems; (3) limitations in usual role activities because of physical health problems; (4) bodily pain; (5) general mental health (psychological distress and well-being); (6) limitations in usual role activities because of emotional problems; (7) vitality (energy and fatigue) and (8) general health perceptions.[6] The survey can be administered by a trained interviewer or completed by the subject himself. Translations into Afrikaans and Xhosa were made and validated as described below.

Study design

The study comprised three parts:

1. Establishment of normal values for the South African population: The SF-36 was administered to healthy non-medical hospital personnel (domestic workers, porters and administrative staff) from the three racial groups of both sexes, between the ages of 20–40.

2. Validation of the translated instruments: The validity of each of the translated instruments was examined by a test–retest procedure to ensure reproducibility, and by patient interview to show concurrent validity.

Reproducibility: HIV-subjects completed the translated SF-36 during a clinic visit. A second identical questionnaire was given to them to complete and return 7 days later. The responses on the two questionnaires were compared and κ statistics calculated.

Concurrent validity: A trained research nurse interviewed the patients and completed an additional questionnaire based on their responses. This was compared with the patient-completed questionnaire and κ statistics calculated.

3. Cross-sectional survey: The SF-36 was administered to eligible patients attending the HIV clinic between April 1993–May 1994. Clinical stage according to WHO criteria[8] and CD4 lymphocyte counts were recorded. Where possible, the patients completed the questionnaire themselves, if not, it was administered by the research nurse.

Exclusion criteria were acute or terminal illness, impaired mental status, first clinic visit and prison residence. Patients on secondary prophylaxis for opportunistic infections were included in the study. The HIV clinic database was used retrospectively to identify all eligible patients visiting the clinic during the study period. Those who completed the survey ('responders') were compared with those who did not ('non-responders') to confirm that a representative sample of the clinic population had been surveyed.

Statistical analysis

Scores for each MOS scale were derived from the subjects responses on the SF-36 and transformed to a 0–100 scale, with a higher score representing better function. The reliability and concurrent validity of the translated instruments were assessed by calculating weighted κ statistics[9] for individual questions and median differences between overall scores for each scale.

One-way ANOVA was used to examine the effect on MOS score of race/gender (six groups) in control subjects and of race/gender, WHO clinical stage and CD4 count in HIV subjects. In the HIV subjects, the effect of race/gender was examined within each disease stage and with all subjects grouped together to increase statistical power. Similarly, the effect of clinical stage was assessed within each race/gender group and with all races and genders together. WHO stages 1 and 2 were combined for analysis to increase the statistical power. CD4 counts were grouped into >400, 200–400 and <200.

'Responders' and 'non-responders' were compared using the χ^2 test to determine if there were differences in the proportions sampled with regard to gender, race and clinical stage of disease.

Results

Control data

One hundred and fourteen healthy subjects were surveyed. Forty-nine per cent completed the questionnaire in English, 32% in Afrikaans and 19% in Xhosa. The mean scores for each MOS scale are shown for the different racial groups and genders in Table 1.

ANOVA showed that the race/gender groups were not uniform in their performance on any scale except vitality. Black females scored significantly lower on all scales except physical function, where Mixed race males recorded the lowest scores. When black females were excluded, the groups were found not to differ significantly except for poorer physical function in Mixed race males.

Validation data for the translated questionnaires

Reproducibility: Thirty subjects (16 Afrikaans, 14 Xhosa) completed two translations of the SF-36 a week apart. The overall scores for each scale were compared and the median differences are shown in Table 2. The median weighted κ for individual questions in the Afrikaans version was 0.60 (range= 0.15–1.0), and in Xhosa 0.53 (range=0–1.0). Kappa values for questions referring to vitality were poor in both translations (Afrikaans mean=0.32, Xhosa mean=0.43), as were some of the questions referring to mental health and general health.

Concurrent validity: Interview data from 45 subjects (25 Afrikaans, 20 Xhosa) was compared with their independent responses on the SF-36. The overall scores for each scale were compared and the median differences are shown in Table 2. The median weighted κ for individual questions on the Afrikaans version was 0.73 (range 0.04–0.98), and 0.57 (range 0.17–90) on the Xhosa. The questions used to calculate the vitality score performed poorly with a mean κ of 0.40 for the Afrikaans translation and 0.41 for the Xhosa.

Patient data

One hundred and thirty-four HIV patients were surveyed (53 WHO stage 1 and 2, 51 stage 3 and 30 stage 4). Twenty-six per cent of patients visiting the clinic were not eligible to be included in the survey.

Table 1. Mean MOS scores (95%CI) for control subjects by race and gender

	White M (*n*=21)	White F (*n*=15)	Mixed M (*n*=19)	Mixed F (*n*=18)	Black M (*n*=21)	Black F (*n*=21)
PHYS	89 (78–99)	94 (82–100)	64 (53–75)	81 (70–92)	88 (78–98)	73 (62–83)
ROLE	88 (77–99)	90 (77–100)	80 (69–92)	97 (85–100)	86 (75–97)	68 (57–79)
PAIN	85 (73–96)	86 (72–99)	79 (68–91)	80 (67–92)	75 (64–86)	55 (43–66)
GEN	75 (67–83)	76 (67–86)	78 (70–87)	74 (65–83)	67 (59–75)	61 (53–69)
VIT	68 (60–76)	57 (48–66)	74 (66–82)	65 (57–73)	72 (65–80)	66 (58–74)
SOC	87 (77–96)	76 (65–88)	81 (71–92)	81 (71–92)	84 (74–93)	63 (53–72)
EMOT	77 (62–92)	73 (55–90)	83 (68–99)	90 (74–100)	77 (62–92)	52 (37–66)
MENT	78 (71–85)	70 (61–79)	83 (75–91)	75 (67–83)	80 (73–87)	65 (57–72)

PHYS=physical health, ROLE=role–physical, GEN=general health, VIT=vitality, SOC=social functioning, EMOT=role–emotional, MENT=mental health

Table 2. The median differences between MOS scores on each scale in test–retest studies

	Afrikaans reliability	Afrikaans validity	Xhosa reliability	Xhosa validity
PHYS	5	0	5	2.5
ROLE	0	0	0	0
PAIN	8.5	0	11.5	1
GEN	11.5	5	8	5
VIT	10	7.5	12.5	12.5
SOC	0	0	0	12
EMOT	0	0	0	0
MENT	8	8	12	12

PHYS=physical health, ROLE=role–physical, GEN=general health, VIT=vitality, SOC=social functioning, EMOT=role–emotional, MENT=mental health

Table 3. Mean MOS scores (95% CI) related to CD4 lymphocyte count

	Control (n =114)	CD4>400 (n =32)	CD4 200–400 (n =44)	CD4<200 (n =58)
PHYS	81 (76–86)	67 (57–76)	71 (63–79)	70 (63–77)
ROLE	84 (78–91)	59 (47–72)	54 (43–65)	46 (37–55)
PAIN	76 (70–81)	60 (50–71)	56 (47–65)	60 (52–67)
GEN	72 (68–76)	44 (37–52)	51 (44–57)	43 (37–49)
VIT	68 (64–72)	52 (45–60)	53 (47–59)	48 (42–54)
SOC	79 (74–84)	64 (54–73)	63 (55–71)	59 (52–66)
EMOT	75 (68–82)	54 (40–67)	47 (35–58)	49 (39–59)
MENT	75 (72–79)	58 (51–65)	61 (55–67)	61 (56–66)

PHYS=physical health, ROLE=role–physical, GEN=general health, VIT=vitality, SOC=social functioning, EMOT=role–emotional, MENT=mental health

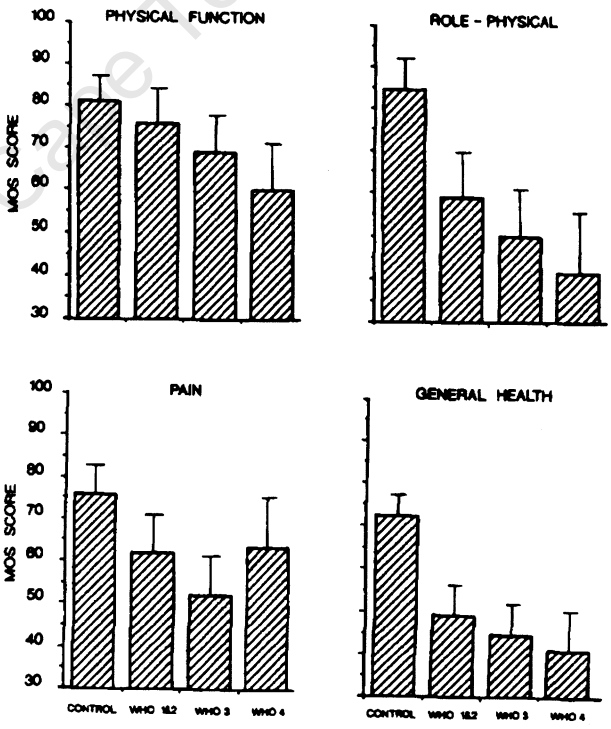
Of the remainder, no differences were found with respect to gender, race and clinical stage of disease between 'responders' and 'non-responders', thus, the 134 patients surveyed were representative of all eligible patients attending the HIV clinic during the study period.

Forty-three per cent of questionnaires were completed in English, 34% in Afrikaans and 23% in Xhosa. Only five questionnaires (0.4%) were completed by the research nurse. The patient group comprised 42 Whites (3 female), 49 Mixed race (14 female), 43 Blacks (28 female). Mean CD4 lymphocyte counts in WHO stages 1, 2, 3 and 4 were 367, 382, 257 and 93 respectively. The mean age was 32.5 and this did not differ significantly between the six race/gender groups; age was therefore not considered further in the analysis.

Physical function was the only MOS scale to be affected by race/gender in HIV-infected subjects; mixed race males and females had lower scores (p<0.05). The mean scores (±95%CI) for each scale related to WHO stage are shown in Figures 1 and 2. HIV subjects scored significantly lower than controls on all scales (p<0.001). The majority of the overall decline in function had occurred by WHO stage 1 and 2 (50% on pain, 51% role-emotional, 55% social function, 60% role-physical, 62% vitality, 74% general health and 88% mental health). Physical parameters showed a trend towards a linear decline with disease progression, but this did not reach significance. MOS scores on the other scales did not decline significantly with advancing disease. Similar findings were evident when MOS scores were related to CD4 lymphocyte count (Table 3).

Twenty-five per cent and 26% of stage 1 and 2 subjects scored zero on the role–physical and role–emotional scales, respectively. There were few zero scores on the other scales.

Figure 1. The relationship between mean MOS scores (+ 95%CI) on physical function, role–physical, pain and general health scales and WHO clinical stage of disease

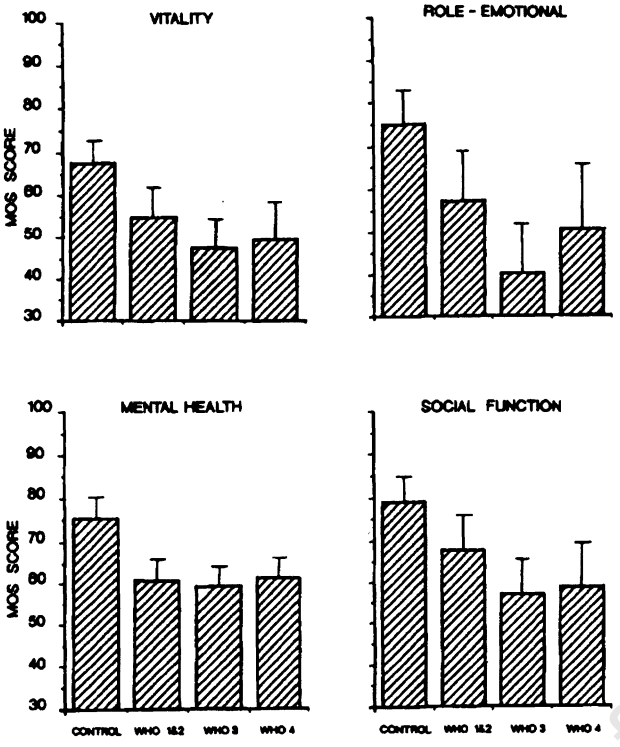


Discussion

The instrument

Quality of life is a broad concept that includes not only functional status and well-being, but also involves other aspects not directly related to health, such as income, freedom and environment. Increas-

Figure 2. The relationship between mean MOS scores (+95%CI) on vitality, role–emotional, mental health and social functioning scales and WHO clinical stage of disease



ingly, health is being evaluated not only in terms of life expectancy but also quality of life remaining. Furthermore, functional status may be a strong independent predictor of survival in HIV-infection.[5]

An instrument designed to measure quality of life in one country may not be appropriate for use in another as concepts of health may vary across cultures and these cultural differences can adversely affect the instruments validity. However, the SF-36 was able to differentiate between healthy and HIV-infected individuals on all scales and appeared to be a satisfactory measure of quality of life in the South African HIV population.

Translating an instrument can also cause problems as it may be interpreted differently in the new language. Validation studies of the Afrikaans and Xhosa translations of the SF-36 demonstrated good overall reliability and concurrent validity on most scales. However, the κ statistics relating to questions on the vitality scale, in particular, were not acceptable. This was probably due to the complexity of these questions in a relatively uneducated population. Further work is being carried out on the translations according to international guidelines.[10]

Control data

Previous MOS data have shown that men tend to report slightly better health than women on all measures except health perceptions and Non-whites report poorer health perceptions, social functioning and more pain. In addition, education and income influence MOS scores.[11] Thus, the lower scores reported by Black females in our study are in line with previously reported data for females and Non-whites, but could also be attributable to poorer socioeconomic status which was not directly controlled for in the study. There are obviously limitations to using a small, fairly select control population, but it was beyond the scope of this study to match patients and controls for socioeconomic factors. However, socioeconomic status and race are still interdependent in South Africa and the majority of patients seen at the hospital are of lower socio-economic class and would be comparable with the controls selected. A possible confounding factor is that controls were not tested for HIV; this might be particularly relevant in Black females who currently have the highest incidence of HIV in Cape Town. However, given its political history, quality of life issues in South Africa might be expected to differ between racial groups and Black females probably have poorer quality of life. There was no obvious explanation for the reported poorer physical function of Mixed race males, but this was consistent in both control and HIV subjects.

Patient data

We have shown that all aspects of quality of life are affected in 'asymptomatic' HIV-infection. This early impact was maximal on emotional and psychological factors, whereas physical function showed a more linear decline with disease progression. One might expect quality of life to deteriorate as a person becomes more symptomatic, and quality of life in HIV-infection has been shown to be related to clinical stage of disease in some studies.[4,5] Wu showed that, compared with AIDS-related complex, asymptomatic HIV patients reported better overall health, physical function, role function and less pain,[3] and Burgess[12] demonstrated deterioration in measures of physical health with disease progression. Our data also suggest that physical function declines with advancing disease and the lack of significance may be due to a type 2 error. Furthermore, all subjects who were acutely ill were excluded from the survey and since most of them were clinically WHO stage 3 or 4 our results

represent a conservative estimate of the decline in function with advancing disease. Possible declines in the physical and emotional aspects of role function with disease progression might have been missed due to the relative insensitivity of the SF-36 at the lower end of these scales. This has been noted with previous MOS instruments in HIV patients[2,12] and additional items to improve sensitivity in the lower ranges of the role functioning scales would be beneficial.

The majority of literature on the psychiatric aspects of HIV disease comes from the USA and Europe, and concerns homosexual and bisexual men; it therefore may not apply to the largely heterosexual HIV population in South Africa. Several studies have shown an increase in psychiatric morbidity in early symptomatic HIV disease.[13] HIV-infection clearly had an early impact on psychological well-being in the Cape Town HIV population with little further deterioration demonstrable on progression to AIDS. However, this study did not address the nature of psychological disturbances present and these may well be different at different stages of disease.[13]

Race and gender appeared to have little effect on quality of life once HIV-infection was present with a significant difference between groups being detected only on one of the eight scales measured. Despite the differences in the control population, Black females with HIV-infection scored comparably with the other race/gender groups. It is possible that the overwhelming effect of HIV-infection on quality of life might have masked an effect of race or gender and that a statistical difference could have been found with larger numbers, but it seems unlikely that this would be of clinical importance.

Using a comprehensive instrument, we have shown that HIV-infection impacts early on all aspects of quality of life and that race-related differences in quality of life do not appear to be important in the South African HIV population. With the emphasis in the South African Health Service now being placed on primary care, it is important not to neglect the needs of patients with early HIV disease who have considerable morbidity, especially psychologically, and to make provision to support them at a primary care level.

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Quality of Life in HIV Infection

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HIV infection gives rise to incurable, debilitating disease in young persons and treatments capable of prolonging survival are often associated with toxicity. Quality of Life (QOL) assessment is therefore important in the evaluation of clinical interventions in HIV-infected patients. It also has a role in determining the needs of individuals and directing healthcare resources. A wide range of QOL instruments is available and choice of instrument should be guided by the information required. As would be expected, poorer QOL is correlated with the presence of disease-related symptoms and advancing WHO clinical stage of disease. Psychological dysfunction, however, is evident from the time of diagnosis and should not be overlooked.

Key words: AIDS; CMV; functional status; HIV; MOS; Quality of Life (QOL); well-being; zidovudine

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With no prospect of a cure for human immunodeficiency virus (HIV) infection in the near future, current management of HIV-infected persons is directed towards prolonging survival and maintaining Quality of Life (QOL). HIV infection, unlike most other chronic medical conditions, affects young otherwise healthy adults causing slowly progressive disease. Many HIV-infected persons remain asymptomatic for years (mean time to AIDS 10 years). With advancing disease, however, constitutional symptoms (fever, night sweats, weight loss), diarrhoea, dyspnoea, cognitive and neurological impairment become more prevalent. Psychological dysfunction, on the other hand, is prominent from the time of diagnosis, as not only do patients have to come to terms with the fact that they have an incurable disease, but they also have to contend with the stigma attached to the diagnosis (1).

Care must be taken in interpreting QOL data from HIV-infected persons as they are a sociodemographically diverse group. QOL measures designed for use in Western HIV populations may not be appropriate for use in other parts of the world, as concepts of health vary across cultures. Furthermore, translation of an instrument can cause problems as it may be interpreted differently in the new language. Instruments should therefore be validated in the HIV population under study. For these reasons, there has been a lack of QOL data from HIV patients in non-industrialized countries.

This review discusses the role of QOL assessment in HIV infection and QOL data reported from cross-sectional, longitudinal and interventional studies in HIV-infected persons.

ROLE OF QOL ASSESSMENT IN HIV INFECTION

Clinical trials

Health status has become an increasingly important outcome measure in HIV-related clinical trials, especially where 'asymptomatic' persons are studied who have a relatively low incidence of hard endpoints, such as disease progression or death. Toxicity may be poorly tolerated by patients who are relatively well. Therapies that can be of benefit in prolonging life in HIV-infected persons are often associated with unpleasant side effects and may increase longevity at the expense of QOL. Thus, QOL measures can give information which will help determine not only the acceptability of the treatment to the patient but also the optimal effective dose of a medication.

In a placebo-controlled trial of high-dose zidovudine in early 'symptomatic' HIV infection, zidovudine delayed progression of disease but QOL data documented impairment in all dimensions of well-being (2). Subsequently a lower dose of zidovudine was shown to be associated with at least as good survival and less toxic side effects (3).

Individual assessment

In the individual HIV-infected patient QOL measures can be used to determine specific areas of dysfunction which may be amenable to treatment. QOL data from clinical interventions can also be used to help patients make informed decisions about their treatment. This is of particular relevance with regard to a condition like cytomegalovirus retinitis, which causes blindness if untreated, but effective therapy until recently required life-long intravenous administration of medications with adverse side-effect profiles. A questionnaire has been developed that measures visual function, treatment impact and QOL in patients with CMV retinitis to address this problem (4).

Healthcare planning

QOL data may assist in healthcare planning by providing information that identifies groups of HIV patients at particular risk of developing problems, so that interventions can be

timeously targeted at these groups. For instance, psychological stress is well documented in 'asymptomatic' HIV disease (1) and early psychosocial intervention has been shown to improve QOL (5), thus it is appropriate to provide counselling and social support for HIV-infected persons from the time of diagnosis.

In resource-poor countries it is important to ensure that the limited funds available for healthcare are directed to the areas of greatest need where there is potential for benefit. Assessment of functional status following treatment of AIDS-related illnesses, such as cryptococcal meningitis, will document whether patients are returned to a functional lifestyle. A decision may then be made to provide treatment for these conditions rather than for those where the functional outcome is less good.

INSTRUMENTS FOR MEASURING QOL IN HIV

There are many different concepts related to QOL and it is important to define what aspects of health status need to be assessed in a specific situation (6). Several standardized, generic measures of health status have been developed for use in chronic medical conditions and some of these have been validated in the HIV population, e.g. the Medical Outcome Survey instruments MOS SF-36 and MOS-20 (7, 8). However, these instruments were not designed for use in this population and may be insensitive to some aspects of QOL that are of particular importance to HIV-infected persons. A Medical Outcome Survey instrument has been developed for use in HIV patients (MOS-HIV) by inclusion of questions relating to energy level, cognitive function and health distress (9) and a number of other instruments have been structured specifically for use in the HIV population. The single-item, observer-dependant Karnofsky Performance Scale has been widely used in HIV-related clinical trials to assess physical dependance but gives no information on well-being (10). Standardized non-QOL measures, e.g. the Hospital Anxiety and Depression Rating scale, can be used for the assessment of specific areas of function. The ideal instrument for use in HIV clinical trials should be short, self-administered, measure the desired aspects of QOL, be sensitive to small changes in function and have been validated in the HIV population under study.

CROSS-SECTIONAL DATA ON QOL IN HIV INFECTION

Studies of QOL in HIV populations must take into account the diverse nature of the persons affected as findings in one group may not necessarily be applicable to another. The strongest predictors of poorer QOL in HIV-infected persons have been found to be the presence of disease-related symptoms (8, 9) and advancing WHO clinical stage of disease (11, 12). Minor differences in health status have been found in HIV-infected persons of different racial origin, gender and age (8, 11). Intravenous drug abuse and lower education level have been associated with lower reported function in all areas (8). Constitutional and neurological symptoms have the greatest overall negative impact on health status, chronic diarrhoea has a serious adverse effect on role function by disrupting ability to work or perform normal daily activities and dyspnoea interferes more with physical than role function (8, 13).

HIV-infected individuals report impairment in all aspects of health-related QOL when compared with healthy persons from the same population (11). Poorer function was evident even in persons with early 'asymptomatic' HIV disease, but was more marked in areas relating to psychological function whereas physical function declined in a more linear fashion with disease progression. Generalized scales that measure psychological distress show little change in psychological function, with advancing disease, but more specialized instruments may show that the nature of the stresses change. For instance, at the time of diagnosis, anxiety, guilt and denial may be prominent, whereas, with the onset of symptomatic disease, adjustment disorders and depression are more common.

'Asymptomatic' HIV patients generally report better QOL than outpatients with other chronic conditions, although they experience more psychological distress. Patients with early 'symptomatic' HIV disease had similar QOL profiles to those with hypertension except for poorer mental health and more health distress (9).

LONGITUDINAL DATA ON QOL IN HIV INFECTION

Knowledge of the natural history of QOL in HIV infection is important in understanding the significance of QOL data related to clinical interventions. The progressive nature of the disease must be taken into account as stabilization of declining function with a particular intervention may represent a significantly better outcome than would have been expected in the natural course of the disease.

There is a paucity of longitudinal QOL data in HIV infection. A prospective study of QOL in the Cape Town HIV population, using the MOS SF-36, documented a significant decline in physical function, general health and physical component score (14) in symptomatic patients (including those with AIDS) at 1 year of follow-up (Table I). Lubeck and Fries were able to demonstrate deterioration in disability, general health, energy and symptoms even in 'asymptomatic' HIV patients at a mean of 9 months follow-up using the AIDS-HAQ (12). Neither study showed any changes in mental health scores with time. Prospective studies of health status in advanced HIV patients are likely to underestimate deterioration due to the high mortality of this group, as follow-up data will only be available for the 'survivors' who are likely to be healthier.

It has been suggested that QOL is an independent predictor of survival in HIV-infected patients. Stanton et al. showed

Table I. Mean MOS scores in symptomatic HIV patients (WHO clinical stages 3 and 4) at baseline and 1 year follow-up

	Baseline	One year	p value*
Physical function	75	64	$p < 0.05$
Role—physical	55	50	NS
General health	49	42	$p < 0.05$
Pain	57	52	NS
Vitality	55	49	NS
Social function	58	61	NS
Role—emotional	53	52	NS
Mental health	64	63	NS
PCS	43	38	$p < 0.05$
MCS	42	43	NS

PCS = physical component score, MCS = mental component score.
*Paired t-test (two-tailed).

that the risk of death was greater in HIV patients who needed assistance with activities necessary for independent living than in those with no functional dependencies (15). However, in the Cape Town HIV outpatient population, MOS scores did not predict survival. Instruments specifically documenting functional dependence may be better able to predict survival than generic measures of functional status and well-being.

CONCLUSIONS

QOL assessment in HIV-infected persons is important as an outcome measure in clinical trials, in determining specific needs of individuals or groups that might be amenable to therapy and in directing the appropriate use of healthcare resources. Instruments need to be adapted and validated for use in the different HIV populations and there is an urgent need for the development of QOL instruments referrable to Third World countries.

A knowledge of the natural history of QOL in HIV infection is necessary to be able to interpret QOL data associated with clinical interventions. The deterioration in physical aspects of QOL associated with advancing HIV infection is largely attributable to disease-related symptoms, but psychological stress is apparent even in early 'asymptomatic' disease.

In the context of the vast scale of the HIV epidemic and the dwindling resources available for healthcare and social support in many countries, it is important to be able to identify areas where intervention can improve function and keep HIV-infected persons independent for as long as possible. QOL assessment will contribute significantly to our ability to do this.

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CD4 and total lymphocyte counts as predictors of HIV disease progression

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Summary

CD4+ T-lymphocyte (CD4) counts are a standard laboratory marker of disease progression in HIV infection, but expense precludes their use in large parts of the world. Total lymphocyte counts (TLC), in contrast, are widely available. We compared CD4 and TLC counts as predictors of developing AIDS or death in 831 HIV-positive out-patients (582 males and 249 females with both homosexual (males, $n=316$) and heterosexual ($n=515$) transmission patterns. The first CD4 count $<200/\mu l$ and first TLC $<1250/\mu l$ predicted similar ($p=0.52$) survival, irrespective of clinical stage. For each clinical stage, a significant difference in progression to AIDS and mortality was predicted by TLC above or below $1250/\mu l$ ($p<0.03$). Survival and progression to AIDS occurred at similar rates in patients with a TLC $<1250/\mu l$ or a CD4 count $<200/\mu l$ ($p>0.1$), and patients with a TLC $>1250/\mu l$ or a CD4 count $>200/\mu l$ ($p>0.5$). A TLC $<1250/\mu l$ preceded the development of *Pneumocystis carinii* pneumonia or cerebral toxoplasmosis in 76% of patients. In this longitudinal study, TLC and CD4 counts were equal predictors of disease progression. A TLC $<1250/\mu l$ could be considered an indication for commencing cotrimoxazole prophylaxis.

Introduction

HIV infection can be monitored by laboratory[1,2] and clinical[3,4] markers of disease progression. The CD4+ T-lymphocyte (CD4) count is considered the best laboratory marker of progression of HIV infection,[1] but lacks uniform reproducibility,[5] is a crude predictor of HIV disease progression when taken by itself,[6] and, because of expense, has limited availability in both resource-poor and developed countries.[7]

In the absence of CD4+ T-lymphocyte counts, the use of total lymphocyte counts (TLC) has been advocated to predict CD4 count[8] and to stage HIV disease.[4,9] The use of the TLC as predictor of CD4+ T-lymphocyte count is limited by the presence of CD4+ T-lymphophenia in up to 30% of non-lymphopenic patients.[8] However, a low TLC was found to predict progression to clinical AIDS.[2,10] This longitudinal study compared total lymphocyte count with CD4 count as predictor of developing AIDS and death in lymphopenic and non-lymphopenic HIV-positive patients.

Methods

Computer-based medical records of Somerset and Groote Schuur Hospital HIV clinics, two principal Western Cape HIV out-patient clinics, were analysed. Patients had been staged clinically (retrospectively from 1984 to 1991, prospectively from 1992 onwards) at each visit according to the WHO clinical staging system,[9] in which stage 4 is equivalent to the 1987 Centres for Diseases Control (CDC) definition of AIDS.[11] Paired CD4 and TLC values ($n=1965$) were available in 831 patients. CD4 counts were determined by flow cytometry and total

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lymphocyte counts by automated blood cell counter. Lymphopenia was defined as a TLC <1250/μl.

Survival was expressed as the Kaplan-Meier estimate of cumulative probability of survival, and was calculated in months from the index visit (first visit at which CD4 or TLC occurred in the defined range) to the date of death (not censored) or last visit (censored). Similarly, AIDS-free survival was calculated from the index visit to the date of initial AIDS diagnosis/death (not censored) or last visit (censored).

Survival curves were created for various degrees of lymphopenia (increments of 250 lymphocytes) and compared for closest fit to the curves of a CD4 count below 200 and 50/μl. Optimal match of the survival curve of a CD4 count <200/μl was achieved by a TLC <1250/μl. Probability of AIDS-free and overall survival was therefore determined for each clinical stage and a TLC above or below 1250/μl or a CD4 count above or below 200/μl. Statgraphics version 6.0 was used to create Kaplan-Meier plots and the log-rank test used to establish statistical difference between survival curves.

Results

Patients of the two HIV clinics represented both homosexual ($n=316$) and heterosexual ($n=515$) transmission pattern, male ($n=582$) and female ($n=249$) sex, and the three local population groups (Whites $n=280$, Blacks $n=339$, and mixed-race $n=212$). Intravenous drug abuse and haemophilia did not occur as risk factors for HIV infection in our patients.

A CD4 count <200/μl occurred in 81% (547/675) of total lymphocyte counts <1250/μl, and a CD4 count >200/μl was present in 80% (1032/1290) of TLC >1250/μl. A total lymphocyte count <1250/μl was 68% sensitive and 89% specific for a CD4 count <200/μl. A TLC >1250/μl or a CD4 count >200/μl predicted the absence of clinical AIDS in 90% and 94% of patients, respectively. Survival of patients of any clinical stage whose total lymphocyte count had declined below 1250/μl was similar to the survival of patients with a first CD4 count below 200/μl (Figure 1). Survival of patients ($n=132$) with a first TLC below 750/μl was not statistically different ($p=0.37$) from patients ($n=146$) who had a CD4 count below 50/μl (45% at 1 year and 20% at 2 years). In lymphopenic patients (TLC<1250/μl) as well as patients with a CD4 count <200/μl, clinical stage was a major determinant of mortality (Figure 2**a**,**b**). Progression to AIDS and death occurred at significantly ($p<0.03$) higher rates in lymphopenic patients than in non-lymphopenic (TLC>1250/μl) patients (Table 1). Mortality and progression to AIDS were not significantly different ($p>0.5$) between patients

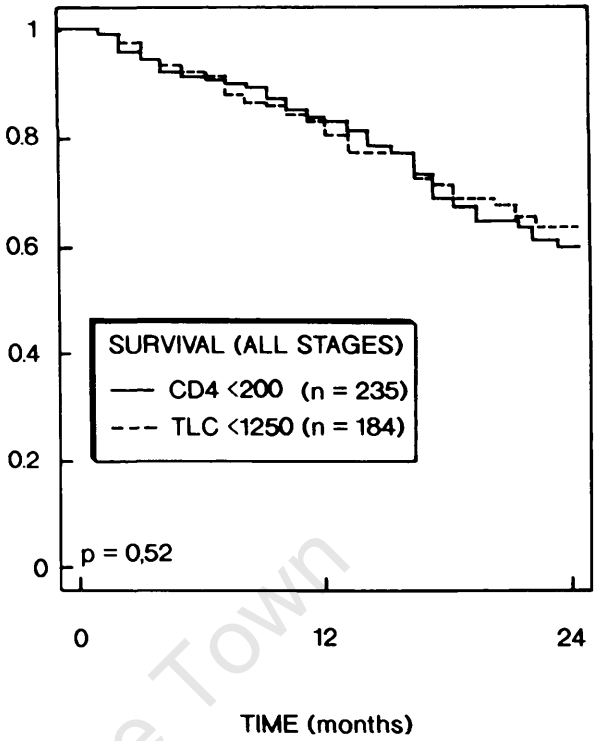

Figure 1. Overall survival for HIV-positive patients from the first CD4 count <200/μl or first total lymphocyte count <1250/μl, regardless of WHO clinical stage.

of similar clinical stage and a TLC <1250/μl or a CD4 count <200/μl (Figure 2**a**–**d**), nor between patients with a TLC >1250/μl and patients with a CD4 count >200/μl ($p>0.1$, data not shown).

Pneumocystis carinii pneumonia (PCP) was diagnosed in 51 patients and cerebral toxoplasmosis in eight. In the 12 months preceding the onset of PCP or toxoplasmosis, a TLC <1250/μl was present in 76% of these patients.

Discussion

The CD4+ T-lymphocyte count is considered the best laboratory marker of progression of HIV infection,[1] and serial CD4 count determinations are commonly used to monitor the degree of HIV-induced immunosuppression. Low CD4 counts are indicative of decreased cellular immunity, and are associated with increased risk of developing AIDS or death. In the absence of CD4 counts, the use of total lymphocyte counts has been advocated to predict CD4 count and to stage HIV disease.[4,8,9] The usefulness of the total lymphocyte count is best evaluated by direct comparison with CD4 count as predictor of end-points such as AIDS and death. This study found CD4 and TLC to be equal predictors of progression of HIV infection. The routine use of total lymphocyte counts rather than CD4 counts would substantially

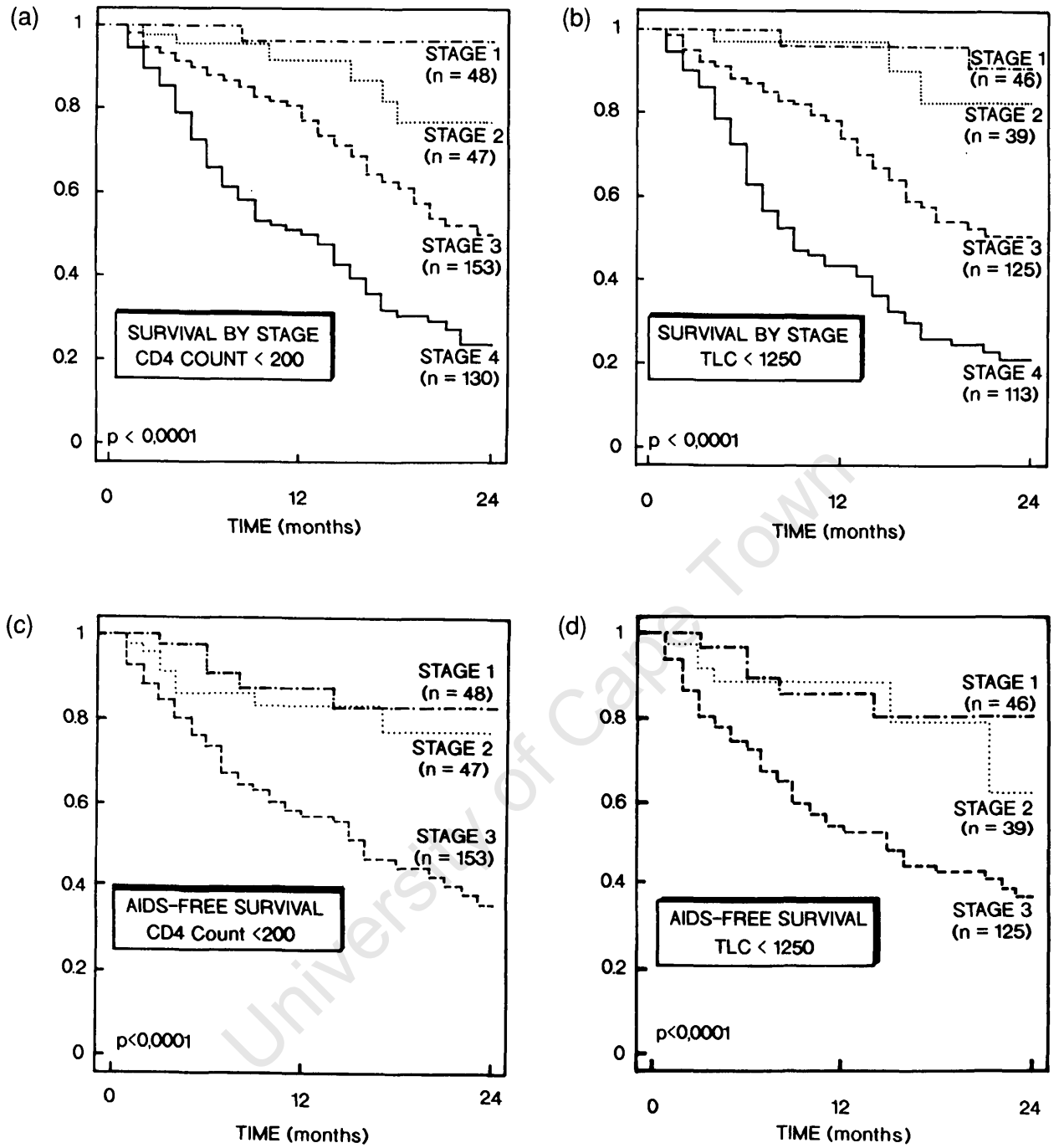

Figure 2. Overall survival and AIDS-free survival for patients with CD4 counts <200/μl and total lymphocyte counts <1250/μl. *p* values represent log-rank comparison of survival curves.

Table 1 One-year Kaplan-Meier estimate of progression to clinical AIDS (WHO stage 1–3) and death (WHO stage 4), stratified by a total lymphocyte count (TLC) above or below 1250/μl

	One-year progression to AIDS			One-year mortality (Stage 4)
	Stage 1	Stage 2	Stage 3	
TLC > 1250/μl	3%	4%	25%	30%
TLC < 1250/μl	14%	12%	46%	55%

reduce the costs associated with managing HIV infection.

The WHO staging system incorporates the use of TLC <1000, 1000–2000 and >2000/μl to replace CD4 counts <200, 200–500 and >500/μl in its laboratory axis.[9] In Rwandan HIV-positive women, few of whom had severe lymphopenia, a TLC above or below 2000/μl had no prognostic value.[12] Our results suggest that a TLC of 1250 rather than 1000/μl should be the equivalent of a CD4 count of 200/μl, and that the presence of a TLC above or below 1250/μl is associated with a significant difference in rates of progression of HIV infection.

In two previous studies, patients of various WHO clinical and/or laboratory stages were rearranged into four 'modified stages', and survival was determined for each 'modified stage'.[4,12] In our patients, TLC (and CD4 counts) added independent, prognostically meaningful information to the WHO clinical stage (Table 1). The stratification of patients by WHO clinical stage and absence or presence of lymphopenia is easily performed and practical for the management of HIV infection in resource-poor countries.

In advanced HIV infection, the total lymphocyte count declines as a result of progressive depletion of CD4+ T-lymphocytes, CD8+ T-lymphocytes and B-lymphocytes.[13] CD8+ T-lymphopenia was found to be an independent predictor of mortality,[14] and the decrease in B-cells may further contribute to the immunodeficient state associated with advanced HIV infection. In our patients, severe lymphopenia (TLC <750/μl) predicted poor survival regardless of clinical stage, and might reflect a high susceptibility to opportunistic infections. In one study, systemic *Mycobacterium avium* complex infection was restricted to patients with severe lymphopenia (mean 540/μl).[15]

Cotrimoxazole is effective prophylaxis against toxoplasmosis, PCP and bacterial infections, and is recommended for all patients with CD4 counts <200/μl.[16] Although PCP is less common in Africa, toxoplasmosis and bacterial infections are major causes of mortality.[17] As lymphopenia preceded the development of PCP or toxoplasmosis in 76% of our patients, a TLC <1250/μl could be considered a criterion for instituting cotrimoxazole prophylaxis.

A TLC >1250/μl was only 4% less sensitive than a CD4 count >200/μl as a predictor of the absence of clinical AIDS. Using lymphopenia rather than CD4 T-lymphopenia as a criterion for commencing cotrimoxazole prophylaxis may thus select a slightly smaller group of patients at risk for developing PCP. Although we have shown the total lymphocyte count to be equal to the CD4 count for overall prognosis, its usefulness in individual patients as a criterion for commencing prophylaxis needs to be studied prospectively.

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AIDS in Africa

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HIV has infected more than 10 million people in sub-Saharan Africa with prevalence rates of up to 30% reported from some countries. Adult transmission of HIV in Africa is mainly heterosexual and over half of new infections are in women. About 40% of infants born to HIV-positive mothers are themselves infected. Diarrhoea occurs in 90% of African AIDS patients and 'slim disease' (prolonged diarrhoea and wasting usually due to coccidian parasites) is pathognomic of AIDS in Central Africa. Dual infection with HIV and tuberculosis is a major problem. African AIDS patients appear to succumb to virulent pathogens, especially *Mycobacterium tuberculosis*, before they become sufficiently immunosuppressed to develop the opportunistic infections typically associated with advanced HIV disease in developed countries.

Key words: Africa; AIDS; coccidian parasites; diarrhoea; HIV; opportunistic infections; 'slim disease'; tuberculosis; wasting

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In 1994 the World Health Organization (WHO) estimated that the human immunodeficiency virus (HIV) had infected more than 16 million adults worldwide and that this figure would increase to 30–40 million by the end of the century, of whom 90% would be in developing countries (1). Sub-Saharan Africa has been the site of nearly two-thirds of all HIV infections to date.

The HIV epidemic in Africa has important differences from that in the USA and Europe. This review highlights some of these differences and focuses on the particular clinical problems encountered in sub-Saharan Africa.

EPIDEMIOLOGY OF HIV-1 AND HIV-2

The origin of HIV-1 is unproven, but it is closely related to other retroviruses present in tropical Africa, the simian immunodeficiency viruses which are endemic in African primates and HIV-2, which is highly prevalent in West Africa. HIV-1 has been present in Central Africa for more than 30 years (2–5) and has spread more recently to West and Southern Africa. Central Africa still has the highest prevalence of infection, with rates of up to 20–30% in women attending antenatal clinics, 50% in people attending sexually transmitted disease clinics and 80% in female prostitutes (6).

In South Africa HIV-1 seroprevalence is increasing rapidly: antenatal surveys in 1992 and 1994 showed a rise in the number of women infected nationally from 0.7% to 8% (7). As in other African countries, the distribution of infection is not uniform tending to be lower in rural and higher in urban areas.

HIV-2 has been present in West Africa for at least 20 years and is still largely localized to this region (8, 9). It appears to be less easily transmitted and less virulent than HIV-1 (10).

Transmission of HIV amongst adults in Africa is mainly through unprotected heterosexual intercourse. The rapid spread of infection has been attributed to several factors, including the number of sexual contacts, multiplicity of sexual partners, availability of prostitution and necessity for men to work away from home for prolonged periods of time. Cultural factors, such as the importance of African women demonstrating their fertility, have undoubtedly played a role. Furthermore, the high prevalence of other sexually transmitted diseases, particularly those that cause genital ulceration, might increase the efficacy of transmission of the virus (11–13). The only country where homosexual transmission has played a significant role is South Africa and the epidemic there, especially in Cape Town, has features of both the North American and African epidemics.

In sub-Saharan Africa, over half of new HIV infections are occurring in women of childbearing age (14). Published rates of mother-to-child transmission of HIV-1 vary from 13 to 45%, but rates of around 40% are typical in Africa (15). This could result in 3–4 million HIV-infected children in sub-Saharan Africa by the end of the decade (6).

GASTROINTESTINAL DISEASE

Diarrhoea and 'slim disease'

Diarrhoea occurs in nearly 90% of patients with advanced HIV infection in Central Africa compared with 30–60% in developed countries (16). It appears to be less common in

Table I. Geographical differences in prevalence of enteric pathogens in HIV patients with diarrhoea

Pathogen	USA (23)	Zambia (24, 25)	Zaire (19)	Uganda (26)
Cryptosporidium	17%	25%	22%	48%
Microsporidium	28%	33%	NA	NA
Isospora	2%	25%	7%	13%
Cytomegalovirus	17%	0%	NA	0%
MAI	10%	3%	NA	1%

USA (23). Endoscopy with biopsy, EM, stool microscopy, culture and parasitology.
Zambia (24, 25). Small-bowel biopsy with EM, stool parasitology and TB culture.
Zaire (19). Stool microscopy, culture and parasitology.
Uganda (26). Endoscopy ± biopsy, stool microscopy, culture and parasitology.
NA. Appropriate investigations to detect pathogen not undertaken.

South Africa, with only 30% of AIDS patients in Cape Town reporting three or more episodes of diarrhoea in the last year (17). Furthermore, prolonged diarrhoea associated with extreme wasting ('slim disease') (18) is present in up to 73% of AIDS patients in Zaire (19) compared with only 11% of a similar African population in Johannesburg, South Africa (20).

Several factors may contribute to the increased prevalence of diarrhoea in Central African HIV patients, amongst them are poor sanitation, contaminated food and water supplies, higher mean ambient temperature, unhealthy methods of food preservation and close proximity to livestock. The lower prevalence of diarrhoea in South Africa may be partly attributable to the fact that the studies reported were conducted in urban areas where basic toilets and water are usually provided, but HIV-related diarrhoea does not appear to be a major problem in rural areas of the country either.

The spectrum of gastrointestinal pathogens encountered in Africa is similar to that found in developed countries (Table I) but coccidian parasites (cryptosporidium, microsporidium and isospora) are responsible for the majority of cases of prolonged HIV-related diarrhoea and disease due to *mycobacterium avium intracellulare* (MAI) and *cytomegalovirus* (CMV) is much less prevalent: this may be as a result of African AIDS patients dying of more virulent infections, particularly tuberculosis, before they are sufficiently immunosuppressed to develop these late-stage infections (21). Shigellae and Campylobacter are commonly isolated from stools but, if treated, do not cause prolonged diarrhoea. Amoebiasis and strongyloidosis do not appear to be more common in HIV-infected persons and although there have been occasional reports of invasive amoebiasis, there is no evidence that strongyloidosis is more severe in HIV-infected persons (22).

Tuberculosis was initially thought to be the major factor in the pathogenesis of 'slim disease' (27), but cryptosporidia, microsporidia and isospora have now been found in up to 77% of cases (19, 24, 26). Microsporidian infections are particularly difficult to diagnose, often requiring facilities not available in African hospitals, and have now been shown to be responsible for many cases of prolonged diarrhoea that were previously considered to be pathogen negative. *M. tuberculosis* is occasionally found in stools of patients with AIDS-related diarrhoea but appears to have originated from pulmonary infections in the majority of cases (25, 28).

African patients with AIDS-related diarrhoea have evidence of more marked villous atrophy and crypt hyperplasia than their European counterparts. This is associated with immune activation, which is probably multifactorial in origin, but may be related to small-bowel parasitic infections in some cases (24). The higher prevalence of protein-calorie malnutrition in Africans would also predispose to the development of more severe enteropathy.

Abdominal pain

Abdominal pain is common at all stages of HIV infection (17). In early HIV infection the underlying pathology is similar to that in the non-HIV population, but with advancing immunosuppression opportunistic infections and tumours become dominant. The aetiology of abdominal pain in AIDS is thus related to the predominant opportunistic infections in the community and differs between Europe and Africa (Table II) with the majority of cases in South African black and coloured patients being due to disseminated TB (29).

Wasting

The AIDS epidemic has hit sub-Saharan Africa at a time of economic crisis associated with drought, crop failure and

Table II. Main causative disorders of abdominal pain in AIDS patients in Europe and South Africa

Disorder	South Africa (29) (n = 3S)	Italy (30) (n = 71)
Disseminated TB	46%	2%
Cryptosporidiosis	17%	6%
CMV enteritis/colitis	17%	11%
HIV cholangitis	9%	8%
Bacterial GI infections	6%	2%
GI non-Hodgkin's lymphoma	0%	17%
Acute pancreatitis	0%	12%
MAI enteritis	6%	9%

NB. More than one diagnosis in some patients.
CMV = *cytomegalovirus*, MAI = *mycobacterium avium intracellulare*.

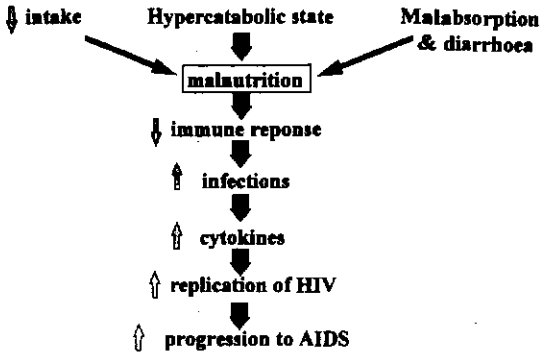

Fig. 1. The interaction between malnutrition and HIV infection.

devastating wars. Malnutrition is therefore prevalent, especially in poor rural communities. Weight loss occurs in 60–90% of AIDS patients in developed countries and is almost universal in Africa. Malnutrition and HIV are inextricably linked (Fig. 1). There is evidence that wasting in AIDS is associated with decreased survival (31), but few data suggest that it is possible to reverse the weight loss and improve the prognosis (32). Recent studies indicate that reduced energy intake at the time of secondary infection is the prime factor rather than increased total energy expenditure (33). Clearly, the high prevalence of diarrhoea and premorbid protein-calorie malnutrition in Africa add to the risk of significant wasting.

TUBERCULOSIS

The World Health Organization estimates that there are between 3 and 5 million persons dually infected with *M. tuberculosis* and HIV, 70% of whom live in sub-Saharan Africa (34). Pulmonary and extra-pulmonary tuberculosis are the commonest 'opportunistic' infections encountered in African AIDS patients in contrast to *Pneumocystis carinii* pneumonia (PCP) in the USA and Europe (35–38) (Table III). Reactivation of latent tuberculosis due to immunosuppression is probably responsible for the majority of cases of HIV-related tuberculosis in Africa. The vast scale of the problem is emphasized by the high prevalence (60–70%) of latent tuberculosis in adult Africans (41, 42). Even in countries with relatively low HIV seroprevalence up to a third of tuberculosis cases are in HIV-infected persons (Table IV). Thus, tuberculosis is a 'sentinel' marker for HIV in Africa (43).

Tuberculosis is not always an easy diagnosis to make, as it is mimicked by other HIV-related diseases. Furthermore, the radiographic appearances of pulmonary tuberculosis in HIV-infected persons are often atypical and may even appear normal in the severely immunosuppressed as they are unable to mount the typical fibrocavitary response (50). Extrapulmonary disease is common in Africa, frequently involving lymph nodes, pleura and pericardium, and dissemination is frequently seen in those with advanced disease.

The effect of tuberculosis on HIV progression is uncertain. Activation of the cellular immune system and cytokine production has been shown to increase HIV replication in vitro, and there is limited data to indicate that morbidity and mortality are increased in African HIV-infected patients who develop tuberculosis (45, 51).

The cornerstone of tuberculosis control remains early case identification and effective chemotherapy. Discussion of the different regimes used in Africa is beyond the scope of this article, but neither primary nor secondary prophylaxis is commonly used because of the concern that non-compliance will increase resistance to available drugs. In contrast to the North American experience, resistance to rifampicin and isoniazid does not appear to be specifically associated with HIV infection.

NON-TUBERCULOUS PULMONARY INFECTION

Pneumocystis carinii pneumonia appears to be less common in African patients but there is considerable variation in reporting with a prevalence of up to 22% in Zimbabwean HIV patients and less than 10% in other Central African countries (52). The prevalence in the Cape Town homosexual HIV population is similar to that reported from the USA

Table III. Frequency of opportunistic infections in AIDS patients in South Africa (Cape Town), Central Africa (Kinshasa, Zaire) and the USA (New York)

Opportunistic infection	South Africa (n = 314)	Central Africa (39) (n = 64)	USA (40) (n = 1822)
Extrapulmonary TB	29%	41%	5%
Pneumocystis pneumonia	23%	2%	67%
Kaposi's sarcoma	18%	16%	15%
Oesophageal candidiasis	16%	31%	17%
Herpes simplex <1 month	12%	NA	1%
Wasting syndrome	12%	13%	3%
Cryptococcal meningitis	7%	19%	5%
Atypical mycobacteria	4%†	NA	8%#
Cerebral toxoplasmosis	3%	11%	10%

†Mycobacteria other than tuberculosis #*Mycobacterium avium intracellulare*.
NB. Pulmonary tuberculosis is not considered an AIDS defining illness in Africa.

Table IV. Relationship between the prevalence of HIV and tuberculosis in sub-Saharan Africa

Country	HIV prevalence of general population	% TB cases HIV infected	Year of study
Uganda (44)	28%†	76%#	1992
Zambia (45, 46)	25%†	60%	1988–1990
Malawi (47±)	23%†	75%#	1990–1994
Ivory Coast (48, 49)	2.4%*	31%	1987–1990
South Africa (7) (Cape Town)	1.3%†	33%#	1994–1995

†Antenatal survey, #Hospitalized patients, *Random adult sample, ±A. D. Harries, personal communication, University of Malawi.

(Table III). The explanation for the lower prevalence in Central African patients is not clear as the organism is ubiqitous in Africa and antibiotic prophylaxis is not widely available. Furthermore, PCP often occurs relatively early in AIDS, in contrast to MAI and CMV.

Pneumococcal pneumonia occurs with increased frequency in African HIV patients and is a significant cause of morbidity and mortality (53). Pulmonary cryptococcosis is seen in HIV infection in Central Africa and, if not treated, may disseminate to cause cryptococcal meningitis (54). Nocardiosis also occurs and may be confused with tuberculosis because of similar clinical and radiological features (52).

OTHER INFECTIONS

Bacteraemia due to non-typhoid Salmonella organisms is very common in African HIV patients and may cause extraintestinal sepsis eg osteomyelitis. It is associated with a high mortality rate (55, 56).

In contrast to North America, MAI has been infrequently isolated from African AIDS patients despite its presence in the environment. Even in the multiracial Cape Town AIDS population, MAI was grown in only 15% of positive mycobacterial blood cultures (57). As discussed above, the low prevalence of MAI may be due to African AIDS patients dying earlier from more virulent organisms, but there is also the possibility that previous exposure to *M. tuberculosis* confers some protection against infection with MAI (22).

Cytomegalovirus, although prevalent in the community, rarely causes significant retinitis or enteritis in Africans (21), but does cause disease in homosexual AIDS patients in Cape Town (29).

NEUROLOGICAL DISEASE

There are very few data on HIV-related neurological disease in Africa and data that have been reported come from small postmortem series. Clinical studies are hampered by the lack of investigational facilities and the poor resources available for treatment. Dramatic increases in cryptococcal meningitis have been documented since 1981 (58, 59). Focal cerebral lesions in Central African HIV patients are reported to be about equally attributable to lymphoma, toxoplasmosis, tuberculosis and cryptococcosis (60, 61) but, in the Cape Town experience, the majority of such lesions are attributable to tuberculosis, often presenting in patients with disseminated disease already on anti-tuberculous therapy.

HIV-RELATED TUMOURS

Kaposi's sarcoma (KS) is endemic in Central Africa, but HIV-related KS behaves more aggressively. Epidemiological evidence has pointed to an infectious aetiology and recently herpes virus (HHV-8) DNA has been found in affected cells but, as yet, its causative role has not been proven (62). Involvement of the gastrointestinal tract with KS may cause bleeding and/or obstruction and infiltration of the lungs may lead to progressive respiratory failure.

High-grade non-Hodgkin's lymphomas are associated with HIV infection in developed countries, but there is a scarcity of data from Africa, which may be attributed to limited resources for investigation and a low postmortem rate. Childhood Burkitt's lymphoma in Africa does not appear to be associated with HIV infection (63).

CONCLUSIONS

While the HIV epidemic has begun to plateau in developed countries, the incidence of new infections continues to rise in sub-Saharan Africa. The epidemic in Africa differs in that it affects young heterosexual adults rather than specific subgroups. The high prevalence of sexually transmitted diseases, together with social and cultural factors, has contributed to the rapid spread of the epidemic in Africa. Many women of childbearing age have become infected and with a vertical transmission rate of about 40% large numbers of HIV-infected children and orphans are inevitable.

Although the spectrum of HIV disease in Africa is similar to that in industrialized countries, infections with pathogenic organisms, particularly *M. tuberculosis*, predominate and opportunistic infections occur less commonly. This is attributable to the high prevalence of virulent organisms, poor access to healthcare and inadequate resources for diagnosis and treatment in Africa. The main burden of disease is related to the gastrointestinal tract and lungs. Coccidian parasites are now recognized to be responsible for

the majority of cases of prolonged diarrhoea and 'slim disease'. Tuberculosis (pulmonary and extrapulmonary) is the most frequent HIV-related illness and is a 'marker' for HIV infection in Africa.

The impact of the HIV epidemic in sub-Saharan Africa is not confined to those who are infected. Displacement of limited healthcare resources by HIV-infected persons has adverse consequences for those with other unrelated illnesses and for primary healthcare, especially amongst children. Furthermore, the struggling economies of the region have been affected by the loss of many HIV-infected professionals and skilled workers. The challenge presented by the human immunodeficiency virus in Africa is daunting, but while efforts to slow the spread of the epidemic through education have had limited success, progress has been made in defining the problems associated with HIV infection and this will allow the resources available to be directed against achievable goals.

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CD4 Decline and Incidence of Opportunistic Infections in Cape Town, South Africa: Implications for Prophylaxis and Treatment

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Objectives: To determine the rate of CD4 decline and the incidence of opportunistic infections (OIs) among antiretroviral therapy–naive South African HIV-infected patients and inform timing of OI prophylaxis.

Methods: We used mixed-effect models to estimate CD4 cell decline by CD4 cell count strata in HIV-infected patients in the Cape Town AIDS Cohort between 1984 and 2000. Stratum-specific OI incidence per 100 person-years of observation was determined using incidence density analysis.

Results: Nine hundred seventy-four patients with 2 or more CD4 cell counts were included. CD4 counts declined by 47.1 cells/μL per year in the stratum with more than 500 cells/μL, 30.6 cells/μL per year in the stratum with 351 to 500 cells/μL, and 20.5 cells/μL per year in the stratum with 201 to 350 cells/μL. Tuberculosis and oral candidiasis were the only OIs that occurred frequently in the stratum with more than 200 CD4 cells/μL. Rates of chronic diarrhea, wasting syndrome, tuberculosis, and oral and esophageal candidiasis increased in the stratum with less than 200 cells/μL, and rates of all OIs were highest in the stratum with 50 cells/μL or less.

Conclusions: CD4 cell count declines were dependent on CD4 strata and can inform timing of clinic visits and treatment initiation in South Africa. Incidence rates of OIs suggest that targeted OI prophylaxis could prevent substantial HIV-related morbidity in South Africa.

Key Words: HIV, opportunistic infections, CD4 cell count, South Africa, prophylaxis

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Despite the apparent similarity in disease survival from HIV seroconversion to AIDS in sub-Saharan Africa and industrialized countries,[1,2] differences in laboratory and clinical markers of progression have not been fully defined. Such differences could have important implications for clinical care and policy in sub-Saharan Africa, yet the research and data necessary to formulate HIV guidelines in the region are lagging far behind that used to create HIV policies in the United States and Europe.[3–6]

Reports from the United States and Europe suggest that in HIV-infected patients not receiving combination antiretroviral therapy (ART), CD4 cell counts decline from 36 to 77 cells/μL per year, with greater absolute declines associated with higher levels of HIV RNA and higher baseline CD4 cell counts.[7,8] This information has been used extensively in Western countries by clinicians making clinical decisions about individual patients and by policymakers in the development of guidelines for the outpatient management of HIV-infected patients.[5] However, it is not yet known whether differences in viral subtypes, human leukocyte antigen types, or other cofactors in sub-Saharan Africa affect the rates of decline, although 2 small studies have suggested slower declines.[9,10]

In addition, the relationship between CD4 cell counts and the risk for specific opportunistic infections (OIs) is poorly understood in sub-Saharan Africa. With high background rates of tuberculosis (TB) and other infectious diseases in the general population, it is important to determine when HIV-infected patients are at greatest risk so that preventive measures and ART can be properly targeted. However, there are limited numbers of large cohorts of HIV-infected patients in sub-Saharan Africa and few that have been followed systematically for long periods.

Our objectives were to estimate the CD4 count declines over time and determine the incidence of OIs in an established cohort of HIV-infected patients in Cape Town, South Africa, in the absence of combination ART and to examine the implications of these findings for the clinical management of HIV disease in the region.

METHODS

Clinical Cohort

We analyzed data from patients enrolled in an observational cohort from 2 public sector clinics affiliated

with the University of Cape Town (Groote Schuur and New Somerset Hospitals), which provided adult HIV care in Cape Town, South Africa. The cohort has been described in detail elsewhere.[11–14] Two thousand eighty-six patients were confirmed to be HIV-infected and enrolled in the cohort between 1984 and the end of 2000. The clinics mainly served indigent communities, with a predominance of heterosexually transmitted infection since 1991.[11]

Demographic information collected on the first visit included date of birth, sex, marital status, race/ethnic background, occupation, number of dependents, sexual preference, and HIV risk factors. HIV diagnosis was confirmed on 2 separate blood specimens by enzyme-linked immunosorbent assay and/or Western blot. Clinical information collected on the first visit and each subsequent visit included weight, diagnosis of OIs and other HIV-related complications, prophylaxis, and other medications dispensed. After 1992, clinical events meeting World Health Organization (WHO) staging criteria were recorded, and a WHO clinical stage was assigned, whereas patients with visits before 1992 were staged retrospectively.[15] Laboratory data were collected approximately every 6 months and included CD4 cell count, blood count with differential, lymphocyte count, hemoglobin level, platelet count, and erythrocyte sedimentation rate. Some patients with a diagnosis of AIDS or with CD4 cell counts of less than 200 cells/μL received cotrimoxazole prophylaxis after 1993.[16] In addition, zidovudine monotherapy was used, although infrequently.

The diagnosis of pulmonary and extrapulmonary TB included both definite TB (culture of *Mycobacterium tuberculosis* or an autopsy diagnosis) and probable TB (a positive smear or a histologic diagnosis together with a clinical response to short-course TB therapy). Diagnosis of other diseases, including chronic diarrhea, cytomegalovirus (CMV) retinitis, cryptococcal meningitis, herpes simplex virus (HSV), esophageal candidiasis, oral candidiasis, *Pneumocystis jiroveci* pneumonia (PCP), prolonged fever, toxoplasmosis, and wasting syndrome were made if definitive diagnosis was available or on standard presumptive grounds as defined by the Centers for Disease Control and Prevention.[17] For this analysis, severe bacterial disease included all WHO stage III bacterial diseases, including pneumonia, bacteremia, pyomyositis, bone and joint infection, meningitis, and empyema, and non-*typhi Salmonella* bacteremia, a WHO stage IV diagnosis. When OIs were diagnosed, standard therapy was available for all OIs with the exception of CMV infection, for which access to antiviral therapy was limited. In the event of death, the date was recorded in hospital records or obtained from close contacts of the patient. If a patient did not attend clinic after a 6-month interval, research staff searched regional death records.

Statistical Analysis

CD4 Cell Count Changes

CD4 cell count changes were examined for the following clinically relevant CD4 count strata: more than 500 cells/μL, 351 to 500 cells/μL, and 201 to 350 cells/μL. Mixed-effect models were used to estimate mean CD4 cell count change and standard deviation for each CD4 stratum over a 12-month period.[18] The model used CD4 decline as the outcome variable, and covariates included time, baseline CD4 cell count, age, sex, and zidovudine monotherapy. To address the issue of differential loss to follow-up between groups (informative dropout), we fit a Cox proportional hazards model using time to dropout as the outcome and CD4 cell count as a time-dependent variable.

Incidence Density of OIs

Patients with 2 or more CD4 cell counts were included in the OI incidence analysis. We used linear interpolation between consecutive CD4 cell counts to estimate the time spent in each CD4 stratum until the occurrence of the OI of interest. The time spent in each CD4 stratum was summed, yielding person-years of observation for each CD4 stratum. In the case for which cotrimoxazole could have a substantial preventative effect (toxoplasmosis, PCP, bacterial disease, chronic diarrhea, prolonged fever, and wasting syndrome), follow-up time was censored at the time of cotrimoxazole initiation. OIs that occurred on the first visit were considered prevalent infections and were not included in the main analysis, and no further follow-up time was attributed to that individual for that particular OI. Follow-up was also censored on the date that patients were recruited away from the natural history cohort and into clinical trials.

We defined the incidence rate of each OI as the number of new cases of a specific infection occurring in a specific CD4 stratum divided by the person-year for that CD4 stratum. Poisson regression was used to examine whether OI incidence rates differed by CD4 stratum and whether incidence rates differed among specific OIs. The analysis was also performed separately for men and women and for different racial/ethnic groups. We also compared the demographic and clinical characteristics of those included in the analyses with those excluded because they had fewer than 2 CD4 cell counts.

RESULTS

Cohort Description

Of the 2086 patients in the cohort, 1766 had 2 or more visits, and 974 of these had 2 or more CD4 cell count measurements and were included in the study. Characteristics of the study cohort are shown in Table 1. Those excluded from the study were not significantly different in age, sex, race, sexual preference, or mean baseline CD4 cell count, although they did have significantly shorter mean follow-up time than those included in the study (18.7 months vs 28.5 months; $P < 0.0001$). The mean initial CD4 cell count for patients included in the study was 307.3 cells/μL (median, 262.0 cells/μL), and on enrollment, 42.1% of patients had WHO stage I HIV disease and 15.0% had stage IV HIV disease. One hundred twenty-five patients received zidovudine monotherapy for some time during the study period.

During follow-up, 306 deaths (31.4% of the study population) were identified from hospital records and regional death records, and 27 patients were recruited from the cohort to clinical trials. Three hundred ninety-eight patients (40.9% of study population) were lost to follow-up

TABLE 1. Demographic and Clinical Characteristics of HIV-1–Infected Patients in the Cape Town Cohort

Characteristics	
Age	32.8 ± 9.2 [14–77]
Sex	
Male	503 (54.6)
Female	419 (45.4)
Race	
Black	478 (51.7)
Mixed race	226 (24.5)
White	220 (23.8)
Risk behavior	
Injection drug use	1 (0.3)
Blood transfusion	8 (2.1)
Multiple partners	129 (33.4)
None	243 (63.0)
Other	5 (1.3)
Sexual preference	
Homosexual	113 (19.9)
Heterosexual	393 (69.3)
Bisexual	31 (5.5)
Unknown	30 (5.3)
Duration of follow-up (mo)	28.5 ± 23.3 [0.1–170.5]
CD4 cell counts per person	3 {2–5} [2–17]
Baseline CD4	307.3 ± 227.5 [1–1351]
	262.0 {133–427}
Baseline CD4 cell count stratum (cells/μL)	
≤50	101 (10.4)
51–200	266 (27.3)
201–350	254 (26.1)
351–500	172 (17.7)
>500	181 (18.6)
WHO stage	
I	410 (42.1)
II	151 (15.5)
III	267 (27.4)
IV	146 (15.0)

Values are given as n (%), mean ± SD, median {IQR}, and [range].
N = 974 (subject to missing data). Percentages were calculated for complete data.
IQR indicates interquartile range.

over the 16-year study period, an average rate of 2.6% per year. Loss to follow-up did not differ significantly by race, although a higher proportion of men were lost to follow-up (47.7%) compared with women (37.0%) ($P < 0.0001$), and those lost to follow-up were slightly younger (31.0 years) than those not lost to follow-up (34.5 years) ($P < 0.0001$). In a Cox proportional hazards analysis, CD4 cell count was not significantly related to dropout ($P = 0.75$ in the age- and sex-adjusted analysis, $P = 0.68$ in the unadjusted analysis).

CD4 Cell Count Decline

CD4 counts declined at the highest absolute rates (up to 47.1 cells per year) in those with more than 500 cells/μL and declined at lower absolute rates in the lower strata (Table 2). Multivariate analysis showed that baseline CD4 cell count had a significant direct association with CD4 decline ($P < 0.0001$), whereas age group ($P = 0.83$), sex ($P = 0.19$), race ($P = 0.15$), and zidovudine monotherapy use ($P = 0.25$) did not.

Incidence of OIs

OIs diagnosed on the first visit were considered prevalent OIs, and most common prevalent OIs included bacterial infections (3.0%), extrapulmonary TB (7.4%), pulmonary TB (11.4%), and oral candidiasis (15.7%). Incidence rate trends of selected OIs are shown graphically in Figure 1; the number of cases and incidence rates (with standard deviation) for each OI by CD4 cell count stratum is shown in Table 3.

Incidence rates were significantly higher ($P < 0.0001$) at lower CD4 cell counts for all OIs, with the highest rates in the stratum of 50 cells/μL or less. In the CD4 stratum with 50 cells/μL or less, the combined incidence of pulmonary and extrapulmonary TB exceeded 20.0 per 100 person-years. Rates of chronic diarrhea, esophageal candidiasis, wasting syndrome, severe bacterial infection, and oral candidiasis were over 10.0 per 100 person-years, and PCP, cryptococcal meningitis, prolonged fever, HSV, and CMV each had rates exceeding 6.0 per 100 person-years. From 51 to 200 CD4 cells/μL, the highest incidence rates were for TB, chronic diarrhea, wasting syndrome, and oral and esophageal candidiasis. TB and oral candidiasis occurred commonly with CD4 cell counts of more than 200 cells/μL and were the only OIs to occur in cell counts of more than 500 cells/μL (not shown), with rates of 0.5 per 100 person-years and 4.1 per 100 person-years.

In a subanalysis of OIs by sex and racial/ethnic groups, there was a significantly higher rate of PCP with 50 CD4 cells/μL or less ($P < 0.001$) in men (14.1/100 person-years) compared with women (1.4/100 person-years) and a trend toward higher rates in whites that was not statistically significant. There were no other significant differences in OI incidence by race.

DISCUSSION

We determined both CD4 cell count declines and incidence rates of OIs in a large, established cohort of HIV-infected patients in Cape Town, South Africa. Yearly CD4 cell count declines ranged from 47.1 cells/μL in the stratum with more than 500 CD4 cells/μL to 20.5 cells/μL in the stratum with 200 to 350 cells/μL stratum. The incidence of all OIs increased significantly at lower CD4 cell count strata. TB and oral candidiasis were diagnosed across all strata and were the only OIs in the analysis to occur commonly in patients with CD4 cell counts of more than 200 cells/μL. The most frequently diagnosed OIs with cell count of lesser than

TABLE 2. CD4 Cell Count Declines by CD4 Count Stratum

CD4 Cell Count Stratum (cells/μL)	CD4 Cell Count Decline (cells/μL) (95% CI)*
>500	47.1 (40.0–54.2)
351–500	30.6 (23.4–37.8)
201–350	20.5 (13.7–27.3)

*Adjusted for age and sex.
CI indicates confidence interval.

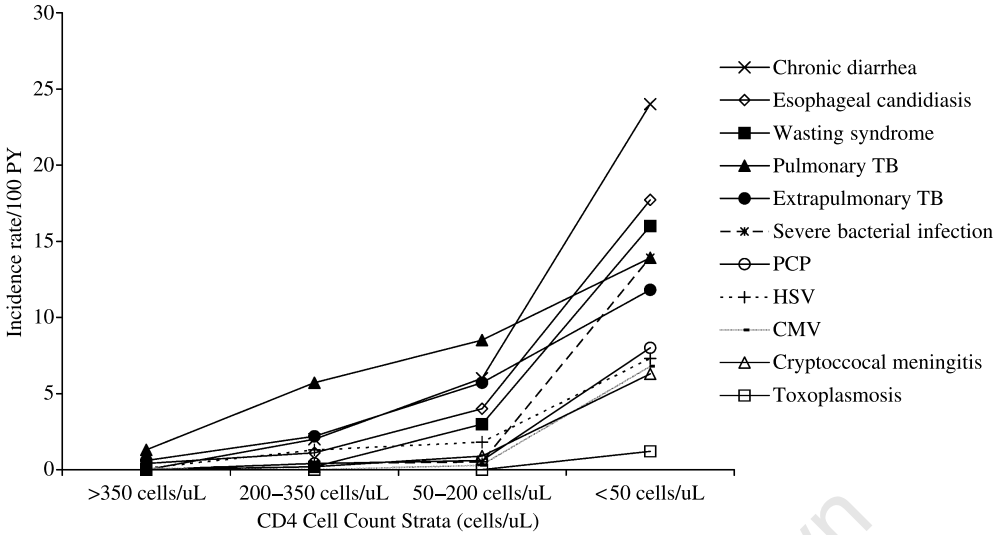

FIGURE 1. Trends in the incidence of OIs by CD4 cell count strata.

200 cells/μL included TB, oral and esophageal candidiasis, chronic diarrhea, and wasting syndrome.

These results come from one of the largest cohorts in the region, which also has the advantage of being an ethnically and sex-diverse group of HIV-infected patients not concentrated in a specific occupation. Although the range of CD4 cell count declines we report overlaps with ranges reported elsewhere in the United States and Europe, mean declines in South Africa (not stratified by viral load) seem generally lower.[7,8,19] In the United States–based Multicenter AIDS Cohort Study, the range of reported CD4 cell count declines ranged from 36 cells/μL (viral load, ≤500 copies/mL) to 77 cells/μL per year (viral load, >30,000 copies/mL), although the mean starting CD4 cell count of 527 cells/μL was higher than that in this cohort.[7] Studies from Zambia and Tanzania also reported lower estimated CD4 cell count declines from 15.0 to 26.9 cells/μL per year in HIV-seroprevalent patients, but were limited by small sample size.[9,10] The findings in the present study suggest that methodologic and sample size considerations may have accounted for some of these earlier reports of substantially slower declines from sub-Saharan Africa, although stratification by viral load is necessary before drawing further conclusions.[9,10]

The approach we took to estimation of OI incidence density has been used in both the United States and Europe and yields clear information about when OIs occur during the natural history of HIV infection.[19,20] Although use of this methodology makes it difficult to compare our results directly with other African studies, several trends were consistent between our studies and others. First, the distribution of TB incidence across all CD4 strata in this study was also seen at TB diagnosis in studies from South Africa and Kenya.[21,22] Likewise, the finding that cryptococcal disease, CMV, and toxoplasmosis occurred almost exclusively in the stratum

TABLE 3. Incidence of OIs Among HIV-Infected Patients in Cape Town, South Africa

OI	≤50 cells/μL		51–200 cells/μL		201–350 cells/μL		>350 cells/μL		P (for Trend)
	Cases	IR (95% CI)	Cases	IR (95% CI)	Cases	IR (95% CI)	Cases	IR (95% CI)	
Oral candidiasis	64	42.7 (32.2–53.2)	122	24.7 (20.3–29.1)	69	15.2 (11.6–18.8)	50	6.9 (5.0–8.8)	<0.0001
Chronic diarrhea	24	24.3 (14.6–34.0)	24	5.9 (3.6–8.3)	9	2.2 (0.8–3.6)	0	0.0	<0.0001
Esophageal candidiasis	38	17.7 (12.1–23.4)	27	4.1 (2.5–5.6)	6	1.1 (0.2–1.9)	3	0.7 (0.0, 1.5)	<0.0001
Wasting syndrome	12	15.6 (6.8–24.5)	10	2.8 (1.1–4.5)	1	0.2 (−0.2–0.7)	0	0.0	<0.0001
Severe bacterial	31	13.9 (9.0–18.8)	3	0.5 (0.0–1.0)	2	0.4 (0.0–0.9)	0	0.0	<0.0001
Pulmonary TB	31	13.9 (9.0–18.7)	52	8.5 (6.2–10.8)	30	5.7 (3.7–7.8)	10	1.3 (0.5–2.0)	<0.0001
Extrapulmonary TB	26	11.8 (7.3–16.3)	36	5.7 (3.8–7.6)	12	2.2 (1.0–3.4)	5	0.6 (0.1–1.2)	<0.0001
PCP	17	8.1 (4.2–11.9)	4	0.6 (0.0–1.2)	2	0.3 (0.0–0.9)	0	0.0	<0.0001
HSV	17	7.3 (3.8–10.7)	12	1.8 (0.8–2.8)	7	1.3 (0.3–2.2)	3	0.4 (0.0–0.8)	<0.0001
CMV	16	6.8 (3.5–10.1)	2	0.3 (0.0–0.7)	0	0.0	1	0.1 (0.0–0.4)	<0.0001
Cryptococcal meningitis	15	6.3 (3.1–9.5)	6	0.9 (0.2–1.6)	1	0.2 (0.0–0.5)	0	0.0	<0.0001
Prolonged fever	5	6.2 (0.8–11.7)	2	0.6 (−0.2–1.4)	0	0.0	0	0.0	<0.0001
Toxoplasmosis	3	1.2 (0–2.6)	0	0.0	0	0.0	0	0.0	—

IR indicates incidence rate per 100 person-years; CI, confidence interval.

with 50 CD4 cells/μL or less was consistent with the low median CD4 cell counts reported for these diseases in South Africa and elsewhere in sub-Saharan Africa.[21,23,24] Although we found relatively high rates of PCP with CD4 counts of 50 cells/μL or less, there was a nonsignificant trend toward a lower rate in blacks.[21,25] It is also possible that delays in PCP diagnosis, while ruling out TB and treating empirically for bacterial disease, may have underestimated the incidence rate with CD4 counts of more than 50 cells/μL.

The results of this study suggest that targeted prophylaxis could have a large impact on reducing OIs, the reduction of which may be a key to reducing long-term mortality in HIV-infected patients.[26,27] Although the data on efficacy of primary prophylaxis of TB are mixed, current WHO guidelines recommend isoniazid preventive therapy in countries with good TB treatment programs in place.[28–31] We found that the risk for TB in South Africa is highest when CD4 cell counts decline to 500 cells/μL or less, which suggests that this CD4 count may be the appropriate threshold if isoniazid is used for primary prophylaxis in this setting. Recent data also suggest a possible role for secondary prophylaxis in patients initiating ART with a clinical history of TB.[32]

Additionally, existing provisional WHO guidelines on cotrimoxazole preventive therapy suggest it should be targeted to HIV-infected persons with stage II or higher HIV disease, those with a CD4 cell count of 500 cells/μL or less, and pregnant women who are in their second or third trimester. These recommendations were based in part on 2 randomized controlled trials of daily double-strength (800/160 mg) cotrimoxazole from Côte d'Ivoire.[4,33] A cohort from Cape Town that overlapped in part with our study population found benefit of single-strength cotrimoxazole only in those with CD4 cell counts 200 cells/μL or less (or TLC ≤ 1250) and/or WHO stage III or IV disease.[16] The present analysis included patients before cotrimoxazole prophylaxis was used and censors follow-up time after its introduction for the incidence of OIs potentially preventable by cotrimoxazole (bacterial infection, PCP, toxoplasmosis, chronic diarrhea, prolonged fever, and wasting syndrome). We found that cotrimoxazole-preventable infections occurred rarely in patients with CD4 counts of more than 200 cells/μL, and thus, this does not suggest a role for cotrimoxazole prophylaxis in this region above 200 cells/μL.

The high rate of cryptococcal disease in the stratum with 50 cells/μL or less and the high case-fatality rate of this infection in other cohorts would seemingly make it a prime target for prevention if prophylaxis was effective and available.[21,23,24] Although no trials of fungal prophylaxis have been reported from sub-Saharan Africa, 2 recent randomized controlled trials from Thailand resulted in a significant decrease in systemic mycoses, of which most were cryptococcal disease.[34,35] Fungal prophylaxis may be appropriate for consideration in South Africa both before widespread availability of ART and as an adjunct when ART is initiated at very low CD4 cell counts.

This study has several limitations. About half of the patients initially enrolled in the Cape Town cohort had less than 2 CD4 cell counts during their follow-up and, therefore, could not be included in the main study. Although these patients had shorter follow-up time, they were not different with regard to their demographics or baseline CD4 cell counts than those included. Conversely, the patients who were recruited away from the cohort to clinical trials may have been healthier, although this number was small. Although loss to follow-up in this study was similar to that in the United States–based Multicenter AIDS Cohort Study, differential losses to follow-up are a concern in longitudinal studies.[36–38] However, we did not find evidence of informative dropout based on severity of disease, as measured by CD4 cell count. In addition, HIV RNA testing was not widely available in Cape Town during the period of this analysis. Therefore, we were unable to report mean CD4 cell count declines stratified by viral load. Lastly, we did not have information on patients' history of OIs before study enrollment. If significant numbers of patients had prior OIs (eg. TB), they would have been at higher risk for recurrence, and we would have overestimated the incidence of those particular OIs.

In conclusion, we report stratum-specific CD4 cell count declines that can be used to inform the development of algorithms for clinical management of HIV in South Africa. We found that rates of OIs in Cape Town increased significantly at lower CD4 cell count strata. Those OIs with the highest incidence at lower CD4 cell counts included TB, bacterial infections, chronic diarrhea, oral and esophageal candidiasis, wasting syndrome, and cryptococcal disease. These results suggest a large potential health gain from maximization of preventive therapies, especially early isoniazid preventive therapy, and cotrimoxazole in patients with 200 CD4 cells/μL or less and prophylaxis against cryptococcal disease with 50 CD4 cells/μL or less. Further work is needed to better quantify the benefits of these interventions in South Africa and to expand the availability of effective therapies.

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Short-term risk of AIDS or death in people infected with HIV-1 before antiretroviral therapy in South Africa: a longitudinal study

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Summary

Background In sub-Saharan Africa, data for short-term risk of AIDS or death, which might inform decisions about when to start antiretroviral therapy (ART), are scarce. Our aim was to investigate these risks in patients who had no access to ART or who were given zidovudine alone.

Methods 6-month risks (%) of death, AIDS, and combined risk of AIDS and death (AIDS/death) were calculated according to CD4-cell count category of less than 200 cells per μL, 200–350 cells per μL, or greater than 350 cells per μL, stratified by WHO clinical stages 1 and 2 combined, 3, or 4 in untreated patients (n=1399) seeking care in tertiary public-sector HIV clinics before widespread availability of ART in Cape Town, South Africa.

Findings Risk of death for WHO stages 1 and 2 was 3·5% for those with less than 200 cells per μL, 2·8% for 200–350 cells per μL, and 1·2% for greater than 350 cells per μL. The corresponding rates for WHO stage 3 were 10·8%, 4·3%, and 4·9% and for stage 4, 22·2%, 10·3%, and 13·8%. 52% (90) of deaths took place in patients without AIDS. 6-month risk of AIDS for WHO stages 1 and 2 was 3·5% for those with less than 200 cells per μL, 1·6% for 200–350 cells per μL, and zero for greater than 350 cells per μL. The corresponding rates for those with WHO stage 3 disease were 17·4%, 7·0%, and 2·2%.

Interpretation In this study, risk of AIDS in patients with a CD4-cell count of less than 200 cells per μL or greater than 350 cells per μL was similar to that previously reported from European cohorts, but was 1·9 times greater for those with CD4-cell counts of between 200 and 350 cells per μL. The high death rate before development of AIDS and a high risk of AIDS in those with CD4-cell counts of 200–350 cells per μL indicate that delay in initiation of ART is associated with increased morbidity and mortality. These findings might help to amend criteria for start of ART in resource-limited settings.

Introduction

WHO estimated in June, 2005, that 4·7 million people living in sub-Saharan Africa were in urgent need of antiretroviral treatment (ART).[1] Several initiatives to achieve large-scale delivery of ART to people infected with HIV in resource-limited countries have been launched. Despite formidable logistical challenges, the number of individuals receiving treatment is steadily increasing.[2] UNAIDS/WHO estimated that 250 000–350 000 deaths were averted in 2005 in low-income and middle-income countries as a result of widened access to HIV treatment.[2] Community-based and hospital-based studies from South Africa have shown that ART is a reasonably cost-effective public-health intervention.[3–5] However, there are limited data on which to base recommendations for when ART should be started in these settings.

Clinical stage of disease, CD4-cell count, and viral load are all well established predictors of prognosis in HIV-infected people.[6–12] Treatment in high-income countries is largely based on the presence of symptoms and on CD4-cell count; additionally, viral load may be used to stratify risk in asymptomatic patients with CD4-cell counts of 200 cells per μL or more.[13–15] Guidelines for the treatment of individuals in low-income countries are based on WHO stage of disease and CD4-cell count.[16] These guidelines are now being revised,[17] but the scarcity of relevant data from resource-poor settings is a hindrance to this process. Thus while acknowledging the limitations of such an approach, some have attempted to model rates of disease progression in resource-poor settings with use of data from European cohorts.[18]

Historically, guidelines have been based on findings of studies that have assessed long-term risk of disease progression. However, findings of the CASCADE collaborative analysis of observational data from 20 cohorts in Europe and Australia underscore the importance of short-term risk assessment of disease progression for informing decisions on when to start ART.[19] No such data exist for individuals living in resource-poor settings. Furthermore, in most guidelines from developed countries, recommendations about when to initiate ART are mainly on the basis of aversion of the risk of AIDS. However, risk of death should also be considered. We therefore undertook this analysis of data from the Cape Town AIDS Cohort (CTAC) study to find out the short-term risk of AIDS, death, and combined risk of AIDS and death according to current CD4-cell count and WHO stage of disease in patients who had either no access to ART or who received zidovudine monotherapy.

Methods

Participants

Details of the CTAC study methods have been described elsewhere.[20,21] The cohort consisted of people with HIV infection living in urban areas who had been referred from a wide range of primary health-care facilities between 1992 and 2005 to tertiary public-sector HIV clinics in Cape Town. Individuals were eligible for inclusion in this study if they were not receiving ART (except for zidovudine monotherapy) and if a current CD4-cell count was available at the start of any 6-month period of assessment. Protocols of the CTAC study were approved by the University of Cape Town clinical research ethics committee.

Procedures

The analyses undertaken in this study were based on the methods described in the CASCADE study, with minor modifications.[19] In separate analyses, we calculated 6-month risk of death, progression to AIDS, and progression to a combined endpoint of a new AIDS-defining disease or death (AIDS/death). In the analysis with death as the endpoint, all eligible patients were included. In the other two analyses, patients already diagnosed with AIDS, as defined by WHO clinical staging criteria,[22] were excluded. Death was identified from patients' records and hospital or municipality death registry.

In three analyses, we divided duration of event-free follow-up into nine strata defined by three categories of CD4-cell count (<200 cells per μL, 200–350 cells per μL, and >350 cells per μL), and stratified further by three WHO clinical stages (stage 1 and 2 combined, stage 3, and stage 4). In every stratum, we recorded the duration of event-free follow-up from the date the patient first entered the respective stratum until the date of progression to another stratum, loss to follow-up, or until death or diagnosis of AIDS were recorded. To limit the analysis to 6 months, event-free follow-up in each stratum was censored after 6 months from date of entry into the respective stratum. Thus any event-free follow-up or endpoint for which CD4-cell count had not been measured in the preceding 6 months was not included in the analysis. However, a patient could contribute further event-free follow-up if they became eligible again at any later follow-up point. The time spent in each CD4 stratum was summed, yielding total patient-years of follow-up for each.

Statistical analysis

We calculated rates for all endpoints by dividing the number of endpoints by the patient-years of follow-up in the respective stratum.

Patients with appreciably different CD4-cell counts in each CD4-cell count and WHO stage stratum were used as the basis for calculations of rates and estimates of 6-month risk of AIDS, death, and AIDS or death. This method was used because every stratum had to be of sufficient size for rates to be calculated with reasonable precision, and these estimates were not adjusted for the potential confounding effect of other variables. Thus we fitted univariate and multivariate Poisson regression models to explore the potential confounding effects of age, sex, socioeconomic status (as defined by Cape Metropolitan Council suburbs composite index[20,21]), and ART monotherapy on risk of AIDS, death, and AIDS or death, using a separate model for each. We considered for inclusion in the final model fitted to generate the 6-month-risk prediction, variables that were significantly associated with the likelihood of AIDS, death, or AIDS or death. To improve the fit of the model, we used the square root value of CD4-cell count instead of the absolute value. We fitted a separate model for each WHO clinical stage. Incidence rates and predicted 6-month-risk (%) rates are presented.

In all models fitted, only CD4-cell count and WHO clinical stage were independently associated with risk of the three endpoints studied. Therefore, only these two variables were considered in the further analyses. For any CD4-cell count, the rate for each endpoint was calculated by WHO clinical stage as: rate=exponential(α+[β√{CD4-cell count}]), where α is the slope and β is the coefficient of the CD4-cell count calculated in the Poisson regression models. Based on the assumption that the rate was constant for 6 months, the 6-month risk (%) was calculated as (1−exp[−0·5 rate]).[12] The predicted rate for each of these endpoints can be calculated using the formulae shown in table 1.

Role of the funding source

The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of all patients recruited into CTAC between 1992 and 2005, 1399 fulfilled the eligibility criteria for inclusion in our analysis. Table 2 shows baseline demographic and

	WHO clinical stage	
AIDS	1 or 2	exp(0·11–0·259√[CD4–cell count]))
	3	exp(0·309–0·152√[CD4–cell count])
AIDS or death	1 or 2	exp(–0·44–0·138√[CD4–cell count])
	3	exp(0·385–0·116√[CD4–cell count])
Death	1 or 2	exp(–1·72–0·08√[CD4–cell count])
	3	exp(–0·895–0·079√[CD4–cell count])
	4	exp(–0·26–0·065√[CD4–cell count])

exp=exponential.

Table 1: **Formulae for calculation of the rate of AIDS, AIDS/death, and death for any CD4–cell count according to WHO clinical stage**

	Number
Male	852 (61%)
Female	547 (39%)
Age, (years)*	32 (27–37)
Socioeconomic status	
High status	913 (65%)
Low status	486 (35%)
Monotherapy ART use	55 (4%)
CD4-cell count (cells per μL)	
Median*	216 (104–392)
<200	668 (48%)
200–350	326 (23%)
>350	405 (29%)
WHO clinical stage	
1 and 2	757 (54%)
3	461 (33%)
4	181 (13%)

ART=antiretroviral therapy. Data are number (%), unless otherwise indicated. *Median (IQR).

Table 2: **Baseline demographic and clinical characteristics of the cohort studied (n=1399 patients)**

clinical characteristics. The study included young adults with a wide spectrum of baseline CD4-cell counts and WHO clinical stages, only a few of whom received zidovudine monotherapy. Median follow-up was 16·6 months (IQR, 8·7–29·3 months). A median of five measurements of CD4-cell count (IQR, 2–11) were available per patient.

Overall, incidence of death was 0·1 (95% CI 0·1–0·2) per patient-year. 14% (n=24) of deaths occurred in patients with WHO stage 1 or 2 disease, 38% (66) in those with WHO stage 3 disease, and 48% (82) in those with stage 4 disease. Thus 52% (52) of patients who died had not been diagnosed with AIDS. Both the rate and 6-month risk of death were significantly higher in patients with advanced immunodeficiency and symptomatic disease than in those in higher CD4-cell-count categories and earlier WHO clinical stages (table 3). In patients with stage 3 or 4 disease, the rate and risk of death did not differ between those with CD4-cell counts of 200–350 cells per μL and those with more than 350 cells per μL.

	Patient years	Number of events	Rate, deaths per patient-years (95% CI)	6-month risk of death, % (95% CI)
WHO stage 1 or 2				
<200 cells per μL	98·2	7	0·07 (0·03–0·15)	3·50 (1·43–7·09)
200–350 cells per μL	158·1	9	0·06 (0·03–0·11)	2·81 (1·29–5·26)
>350 cells per μL	347·1	8	0·02 (0·01–0·05)	1·15 (0·50–2·25)
WHO stage 3				
<200 cells per μL	221·1	47	0·21 (0·16–0·28)	10·08 (7·52–13·19)
200–350 cells per μL	113·8	10	0·09 (0·04–0·16)	4·30 (2·08–7·75)
>350 cells per μL	89·7	9	0·10 (0·05–0·19)	4·89 (2·26–9·06)
WHO stage 4				
<200 cells per μL	145·5	73	0·50 (0·39–0·63)	22·19 (17·91–27·13)
200–350 cells per μL	22·9	5	0·22 (0·07–0·51)	10·34 (3·47–22·40)
>350 cells per μL	13·5	4	0·30 (0·08–0·79)	13·78 (4·11–32·56)
Total				
<200 cells per μL	464·8	127	0·27 (0·23–0·33)	12·77 (10·86–15·21)
200–350 cells per μL	294·9	24	0·08 (0·50–0·12)	3·99 (2·57–5·87)
>350 cells per μL	450·4	21	0·05 (0·03–0·07)	2·30 (1·49–3·44)

Table 3: **Rate and 6-month risk of death according to CD4-cell count and WHO clinical stage**

In 1009 patient-years of follow-up, 111 new AIDS events were identified in patients without previously diagnosed AIDS, yielding an incidence rate of 0·11 events per patient-year (95% CI 0·09–0·13, table 4). The risk of progression to AIDS was greater for patients with low CD4-cell counts than for those with high counts, and was also much greater for those with WHO stage 3 disease than for those with stage 1 or 2 disease (table 4). The 6-month risk of AIDS ranged from zero in patients with a CD4-cell count of more than 350 cells per μL and WHO stage 1 or 2 disease to almost 20% in patients with CD4-cell counts of less than 200 cells per μL and WHO stage 3 disease. The pattern for the rate of progression to AIDS across the CD4-cell count and WHO stage strata was similar to that for risk of death (table 4).

Overall, 179 AIDS or death events took place in the 964 patient-years of follow-up (incidence rate=0·19, 95% CI 0·16–0·22 per patient-year). The combined rate of AIDS/death ranged from 0·02 per patient-year for patients with the highest CD4-cell count and lowest stage, to 0·54 per patient-year in those with the lowest CD4-cell count and stage 3 disease (table 4). The 6-month risk ranged from just greater than 1 to nearly 24 for the same CD4-cell count and stage strata. Both rate and 6-month risk of death increased with more advanced immune suppression and clinical disease (table 4).

The figures 1 and 2 show that both WHO clinical stage and CD4-cell count were strong predictors of the 6-month risk of AIDS, death, and AIDS/death according to our regression models. The risk of all endpoints rose steeply as CD4-cell counts fell from 200 cells per μL (figure 2). However, the risk for each of the endpoints also increased as the CD4-cell count decreased from 350 cells per μL to 200 cells per μL. In particular, the risk of AIDS and AIDS/death increased appreciably in those with WHO stage 3 disease in this CD4-cell count range. Also, for any specific CD4-cell count, risk of death in people with stage 3 disease was about double that for those with stage 1 or 2 disease and risk of death in people with WHO stage 4 disease was about double that of those with WHO stage 3 disease (figure 2).

Discussion

We have derived short-term estimates of the risk of death, AIDS, and combined risk of AIDS or death according to CD4-cell count and WHO clinical stage. The short-term risk of death in individuals without a previous AIDS diagnosis was alarmingly high. The

	AIDS				AIDS or death			
	Patient years	Number of events	Rate, events/ patient-years (95% CI)	6-month risk of AIDS, % (95% CI)	Patient years	Number of events	Rate, events/ patient-years (95% CI)	6-month risk of AIDS or death, % (95% CI)
Stage 1 or 2								
<200	97·1	7	0·07 (0·03–0·15)	3·5 (1·4–7·2)	97·1	14	0·14 (0·08–0·24)	7·0 (3·9–11·4)
200–350	157·3	5	0·03 (0·01–0·07)	1·6 (0·5–3·7)	157	13	0·08 (0·04–0·14)	4·1 (2·2–6·8)
>350	347·1	0	0	0	347·1	8	0·02 (0·01–0·05)	1·2 (0·5–2·3)
Stage 3								
<200	206·9	79	0·38 (0·30–0·48)	17·4 (14·0–21·2)	204·4	110	0·54 (0·44–0·65)	23·6 (19·9–27·7)
200–350	110·7	16	0·14 (0·08–0·23)	7·0 (4·0–11·1)	110·5	24	0·22 (0·14–0·32)	10·3 (6·7–14·9)
>350	89·7	4	0·04 (0·01–0·11)	2·2 (0·6–5·5)	48·4	10	0·21 (0·10–0·38)	9·8 (4·9–17·4)
Total								
<200	304·0	86	0·28 (0·23–0·35)	13·2 (10·9–16·1)	301·5	124	0·41 (0·34–0·49)	18·6 (15·6–21·7)
200–350	268·0	21	0·08 (0·05–0·12)	3·8 (2·5–5·8)	267·5	37	0·14 (0·09–0·19)	6·7 (4·8–9·1)
>350	436·9	4	0·01 (0·003–0·02)	0·5 (0·2–1·0)	395·6	18	0·05 (0·03–0·07)	2·3 (14·5–3·4)

Table 4: **Rate and 6-month risk of AIDS and AIDS or death according to current CD4-cell count and WHO stage**

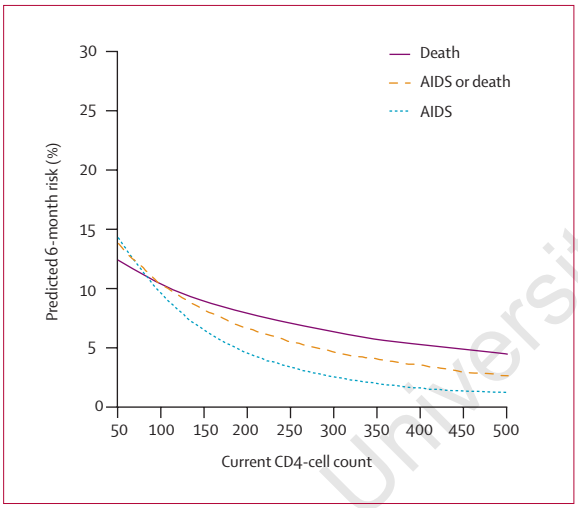

Figure 1: **Predicted 6-month risk of AIDS, death, and AIDS or death in the whole group**

rates of clinical events in people with CD4-cell counts of between 200 and 350 cells per µL was higher than those reported in high-income countries.

Whereas previous studies have used laboratory or clinical markers to measure HIV disease progression in sub-Saharan Africa,[23–30] we focused on assessment of short-term risk of AIDS or death using CD4-cell count and WHO clinical stage. This distinction is important because health-care providers use these criteria when deciding when to start ART. The findings of this study highlight the importance of assessment of not only short-term risk of AIDS, but also short-term risk of death when considering initiation of ART in resource-limited settings, where delayed access to treatment might account for up to two-thirds of early deaths in patients accessing ART programmes.[31,32] Our data are important to the continuing efforts to formulate and refine operational guidelines for scaling up access to treatment in resource-limited countries.

In previous analyses we have shown that WHO clinical stage could be used to identify patients who will benefit most from ART programmes, and that this measure could provide prognostic information independent of CD4-cell count.[20,21] Present WHO guidelines for initiation of ART in resource-limited settings largely recommend use of WHO clinical stage, but also that of CD4-cell counts where available, especially for asymptomatic patients.[16] This study shows that combined use of data for WHO clinical stage and CD4-cell count can be used to accurately assess short-term risk of AIDS and death. Although the cost of routine laboratory testing remains one of the major barriers for increased access to treatment in sub-Saharan Africa,[33] these data nevertheless support moves to expand the use of measurement of CD4-cell count.

This study is strengthened by the use of three important endpoints. Consideration of risk of death and combined risk of AIDS or death when the need for treatment is assessed is especially important in this setting, since the 6-month risk of death is high, amounting to 15% in our cohort.[21] 52% of deaths occurred in individuals without a previous AIDS diagnosis, indicating that WHO stage 3 disease is associated with a high mortality risk in this setting. Analysis of clinical progression that relies on AIDS diagnosis alone might have underestimated the 6-month risk of disease progression by misclassifying some patients as non-progressors for whom recording of an AIDS event was delayed until the next scheduled visit. Misclassification might well happen in AIDS-defining illnesses such as extrapulmonary tuberculosis, in which laboratory confirmation of diagnoses might be delayed.

The patterns of average estimates of short-term AIDS risk by CD4-cell-count categories in this study are in agreement with findings from the CASCADE collaborative analysis of European and Australian cohorts.[19] Comparison

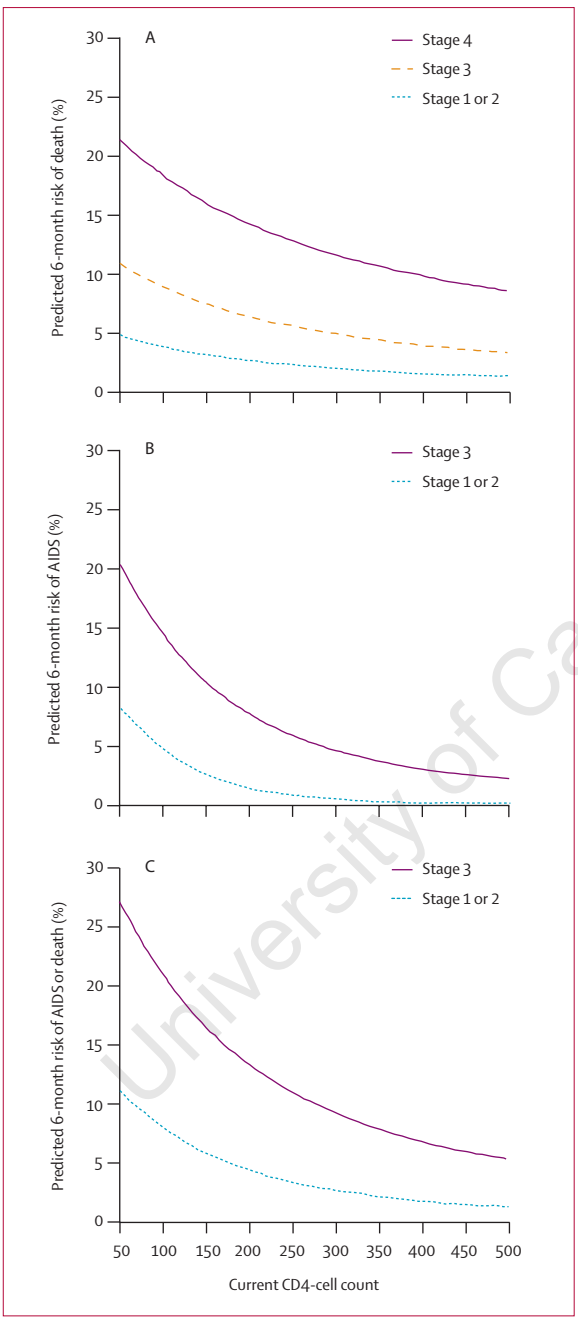

Figure 2: **Risk of death according to WHO clinical stage (A), risk of AIDS according to WHO clinical stage (B), and risk of AIDS or death according to WHO clinical stage (C)**

of the average predicted 6-month risk of AIDS in the two cohorts (adjusted for factors associated with risk of AIDS and calculated for individual CD4-cell counts) show that the risk estimates were strikingly similar. The unadjusted average 6-month estimates of the risk of AIDS of 13·2% and 0·46% calculated in our study are in accord with that of 14·9% and 0·54% that could be calculated in the CASCADE study for patients with low and high CD4-cell counts, respectively.[19] However, in those with a CD4-cell count of 200–350 cells per μL, the risk estimate for AIDS in our cohort was 1·9 times greater than that calculated in the CASCADE study. This difference suggests that in this setting, those with CD4-cell counts of 200–350 cells per μL have a higher risk of morbidity than do similar patients living in high income countries. This finding might be due to differences in the spectrum of HIV-associated disease between resource-limited and high-income countries, or differences in disease progression. This finding might have implications for guidelines for when to initiate ART in resource-limited settings.

This study has some limitations. Information for cause-specific mortality was not available and, therefore, we were unable to identify whether all deaths reported in this study were HIV-related. We included patients who received zidovudine monotherapy, but these patients were only a small proportion of the study population, and the results were unchanged when they were excluded from the analysis. Informative censoring—ie, a greater number of censored observations at an earlier time in one group compared with another, or a greater proportion of censored survival times in patients with a particular range of the explanatory variables) is a well-known issue in longitudinal studies.[34] To take this issue into account, we examined whether our approach might have violated this assumption, by plotting survival times against the explanatory variables included in the Poisson regression models, fitted to construct the formulae used for calculating the predicted 6-month risk estimates of the various endpoints. We showed no discernible pattern suggestive of violation of this assumption. This finding might, as was suggested in the CASCADE study, be attributable to our approach, "censored patients from an observation period and not from the entire analysis".[19] Underestimation of CD4-cell count-specific and WHO stage-specific rates is therefore unlikely.

Risk of AIDS estimates calculated in this study are likely to be generalisable to people infected with HIV from other sub-Saharan African countries in view of the similar spectrum of AIDS-defining illnesses. However, estimates and determinants of risk of death might differ, for example, because of other co-morbid diseases, such as malaria, that are absent in this setting, and because of differing standards of health care.

The difference between the rates of clinical events in our low-income setting and those in high-income countries suggests that delayed initiation of ART in resource-limited settings is associated with increased HIV-associated morbidity and mortality. These data might be useful for refining criteria for initiation of ART in resource-limited settings.

Contributors
All investigators contributed to the study concept, design, and writing of the paper. M Badri did the statistical analysis.

Conflict of interest statement
We declare that we have no conflict of interest.

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The changing pattern of transmission and clinical presentation of HIV infection in the Western Cape region of South Africa (1984-1995)

R Wood, EA O'Keefe, G Maartens

The clinical spectrum of HIV infection in South Africa has not been well documented. This study, at a major HIV referral clinic in Cape Town, describes the changes in clinical presentation of a large adult urban HIV population (n = 1 288) over an 11-year period. From 1984-1991, data were obtained by retrospective chart review and from 1992-1995 were collected prospectively. The attendance of large numbers of patients from each of the three main population groups allows a unique opportunity to compare disease frequency in these groups. During the study period, the change in predominant HIV transmission pattern from homosexual to heterosexual resulted in significant changes in patient demography and HIV presentation. The relative incidence of* Pneumocystis carinii *pneumonia (PCP) declined ($p < 0.01$), while pulmonary and extrapulmonary tuberculosis increased ($p < 0.01$). Wasting syndrome and HIV encephalopathy were noted to occur less frequently in black patients ($p < 0.05$ and $p < 0.01$, respectively). The presentation of AIDS in Cape Town differed significantly in each of the main population groups and from both Western and African reported series. Kaposi's sarcoma, PCP, oesophageal candidiasis and herpes simplex infections were diagnosed more frequently than previously described in South African reports. A knowledge of the local presentation of HIV infection is necessary for the development of appropriate prophylaxis and treatment regimens.

Introduction

South Africa is at present experiencing a rapid growth of the human immunodeficiency virus (HIV) epidemic. By November 1994, the national antenatal HIV seroprevalence was estimated to be 7.6%, while the prevalence in the Western Cape was 1.2%.[1] HIV infection was first recognised in the Western Cape region of South Africa in 1984, and in the following years the epidemic was largely homosexually transmitted (pattern 1). By 1990 the local epidemic had changed to predominantly heterosexual (pattern 2) transmission. Genetic sequence analysis of HIV-1 isolates from patients infected by each transmission pattern, indicates that these are two largely independent epidemics.[2] To date, there has been limited information describing the clinical presentations of HIV in South Africa,[3] and no reports of the changes associated with differing race or transmission pattern.

In 1984, following the identification of the first AIDS cases in Cape Town, a multidisciplinary HIV clinic was started at the New Somerset Hospital (NSH); subsequently a second clinic was opened at Groote Schuur Hospital, Cape Town. The clinic population included patients from each of the three main population groups of the Western Cape.

This study describes the changing demographic profile and clinical presentation of a large diverse urban adult HIV population over the 11-year period from 1984 to 1995.

Materials and methods

The study population consisted of all adult HIV seropositive patients presenting to NSH and Groote Schuur Hospital HIV clinics of the University of Cape Town between July 1984 and November 1995. HIV diagnosis was confirmed on two separate blood specimens by positive screening enzyme immunoassay (EIA) and a combination of enzyme-linked immunosorbent assays (ELISA) and Western blot.

Retrospective clinical chart reviews were performed on patient records prior to January 1992. Demographic details, HIV risk behaviours, laboratory results and clinical data were extracted and entered into a computer database (Epi Info 6.01 Centers for Disease Control, Atlanta, USA, and World Health Organization (WHO), Geneva, Switzerland). From January 1992, data were collected prospectively. Comparisons of disease frequency were made using the chi-squared test.

HIV-related manifestations were classified using the proposed WHO clinical HIV staging system.[4] WHO stage IV equates with AIDS and stage III with AIDS-related complex. $CD4^+$ T-cell counts were measured approximately six monthly, by flow cytometry. Patients with less than 200 $CD4^+$ T-cells/μL were routinely commenced on cotrimoxazole prophylaxis.

Results

During the study period, a total of 8 868 outpatient visits were made by 1 288 patients. AIDS (WHO stage IV) was diagnosed in 342 individuals and 244 deaths were recorded. The mean age of females at presentation was 28.9 years which was significantly lower ($p < 0.01$) than the mean male age of 33.9 years. The changing demographic characteristics of patients presenting between July 1984 to December 1989 and January 1990 to November 1995 are shown in Figure 1. A comparison of AIDS defining diagnoses pre and post January 1990 (Figure 2) demonstrated a significant increase ($p < 0.01$) in extrapulmonary tuberculosis from 5% (1984-1989) to 28% (1990-1995). Similary, pulmonary tuberculosis significantly increased from 4% to 18% ($p < 0.01$), data not shown in Figure 2. Disseminated non-tuberculous mycobacterial infections were diagnosed in 17 patients of

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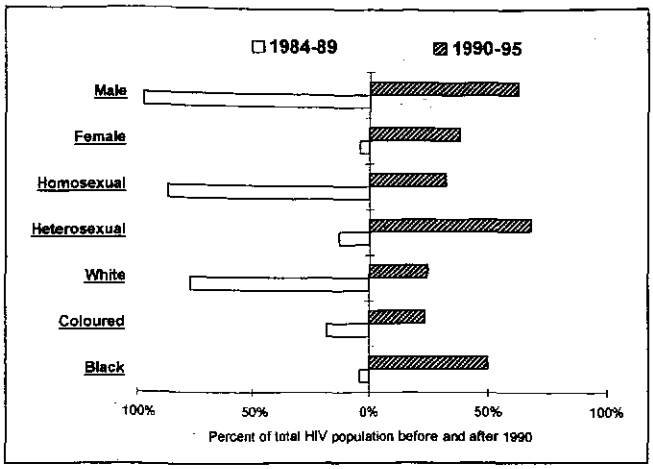

Figure 1: **The demographic characteristics of the HIV outpatient population from 1984 to 1989 involving mainly white male homosexuals (pattern 1) and from 1990 to 1995 showing a marked increased in heterosexual transmission (pattern 2) with involvement of each of the main population groups of the Western Cape**

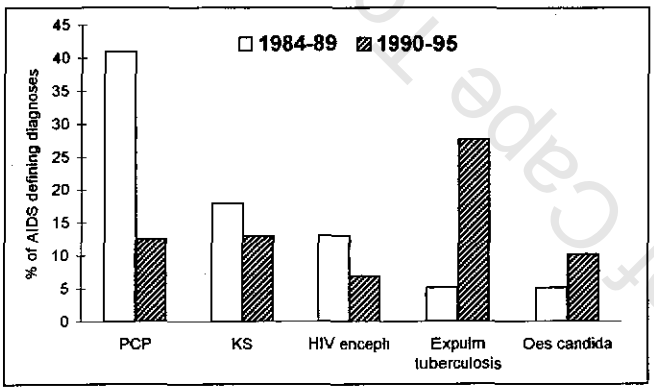

Figure 2: **The 5 most common AIDS defining conditions diagnosed between 1984-1989 and 1990-1995 demonstrating a significant decline in PCP ($p < 0.01$) and increase in extrapulmonary tuberculosis ($p < 0.01$).**
PCP = pneumocystis carinii pneumonia, KS = Kaposi's sarcoma, HIV enceph = HIV encephalopathy, Expulm tuberculosis = extrapulmonary tuberculosis, Oes candida = oesophageal candidiasis

which two were due to *Mycobacterium avium-intracellulare* (MAI). *Pneumocystis carinii* pneumonia (PCP) showed a decline ($p < 0.01$) in incidence from 41% to 12%. Cytomegalovirus infection of the retina and intestinal mucosa occurred only with advanced HIV disease as manifested by a low $CD4^+$ T-cell count (< 50 cells/μL). Unexplained diarrhoea lasting longer than one month was recorded in only 4% of WHO stage III patients.

The frequency with which AIDS diagnoses occurred in each of the three main patient population groups of the Western Cape is shown in Figure 3. Extrapulmonary tuberculosis was diagnosed significantly more frequently in black than coloured and white patients ($p < 0.01$). PCP, Kaposi's sarcoma, HIV encephalopathy and cytomegaloviral (CMV) disease were seen more frequently in white patients ($p < 0.01$). Oesophageal candidiasis also occurred more frequently in the white population ($p < 0.05$).

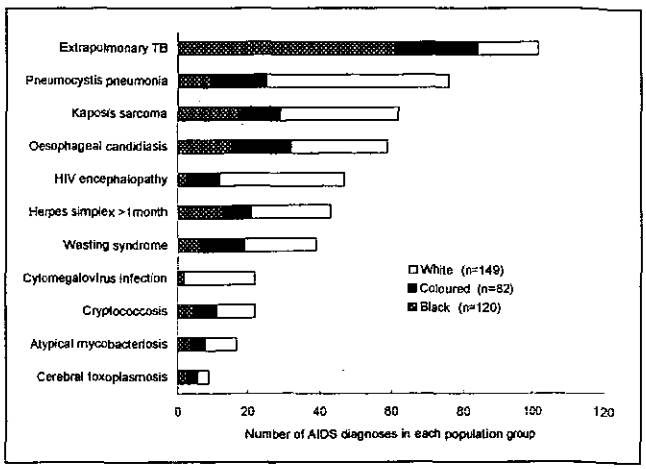

Figure 3: **The total number of AIDS diagnoses (both AIDS defining and subsequent diagnoses) in each of the 3 main population groups of the Western Cape. PCP, KS, HIV encephalopathy and CMV disease occurred more frequently in the white population ($p < 0.01$) while extra-pulmonary tuberculosis was diagnosed more frequently in the black compared to other population groups ($p < 0.01$)**

Discussion

During the study period a change in predominant pattern of HIV transmission was paralleled by changes in HIV presentation. The Western Cape has the highest incidence rate of pulmonary tuberculosis (702/100 000 per annum[5]) on the continent of Africa and this has greatly influenced the local presentation of HIV disease. Pulmonary tuberculosis, which is not included in the WHO stage IV definition of AIDS, has increased together with extrapulmonary tuberculosis, which has become the commonest AIDS diagnosis. The strong interaction between tuberculosis and HIV was demonstrated by a prospective study of NSH inpatients with newly diagnosed tuberculosis and prior unknown HIV status, who had a 33% HIV seroprevalence.[6] The low incidence of disseminated atypical mycobacterial infection amongst our AIDS patients (17 of 342) is in contrast to the experience of developed countries, where up to 40% of AIDS patients develop MAI infection.[7] The low incidence of MAI may result from the high exposure of our patient population to *M. tuberculosis* which may protect against subsequent MAI infection. A reciprocal relationship between tuberculosis and MAI has been shown within the USA where regions with the highest rates of tuberculosis reported the lowest rates of MAI infections.[8]

The high incidence of PCP and Kaposi's sarcoma seen in homosexual white males pre-1990 was similar to that seen in homosexual males in the USA.[9] The decline in PCP after 1990 may reflect the increasing use of primary PCP prophylaxis but is more likely to be related to a lower incidence of PCP in the black and mixed race patients. Although initial reports indicated that PCP was extremely rare in Africa, it has been increasingly diagnosed in African patients.[10,11] Cotrimoxazole acts as a prophylaxis against PCP, bacterial infections and toxoplasmosis[12] and may be responsible for the low incidence of cerebral toxoplasmosis despite evidence of latent toxoplasmosis in 31% of clinic attendees (unpublished data).

The diagnosis of subtle neurological change is difficult

and cultural factors have to be taken into account; however, this should not be a factor when more marked signs of dementia are present. The low incidence of HIV encephalopathy in black patients therefore requires further careful prospective study to confirm this finding.

The low incidence of wasting syndrome (weight loss + fever or diarrhoea) is similar to reports from other areas of South Africa[13,14] but is in contrast to studies from Central Africa where 'slim disease' (weight loss + diarrhoea) is the commonest presentation of AIDS.[15] Although diarrhoeal disease causes significant morbidity in both early and advanced HIV disease in Cape Town,[16] prolonged unexplained diarrhoea was not common. The low rate of unexplained diarrhoea may be related to a lower prevalence of coccidial infections in our population.[6]

Significant changes have occurred in the spectrum of clinical HIV presentation in Cape Town associated with the increase in heterosexually transmitted infection. The frequency of opportunistic infections differ from both Central Africa and developed countries but is dominated by the high prevalence of latent tuberculosis in the community. In each local population group, PCP, Kaposi's sarcoma, oesophageal candidiasis and herpes simplex infections were diagnosed more frequently than previously noted in South African reports.[13,14]

A knowledge of the spectrum of clinical HIV presentations in South Africa is necessary for the rational allocation of resources and the formulation of appropriate prophylaxis and treatment regimens.

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An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa

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Objectives: To determine HIV-1 *env* and *gag* subtypes in male homosexual and heterosexual populations in Cape Town, South Africa.

Design: DNA was isolated from blood originating from 61 patients attending local clinics. Samples were divided according to presumed mode of transmission: male homosexual (n = 26), heterosexual/vertical (n = 32), blood transfusion (n = 1) and unknown (n = 2).

Methods: Proviral HIV-1 DNA was subtyped by heteroduplex mobility assay (HMA) based on the 700 base-pair V3–V5 region of the *env* gene (n = 47) or by sequence analysis of the p17 region of the *gag* gene (n = 33), or both. For HMA, reference plasmids were constructed containing the V1–V5 *env* region sequences (1.2–kb) representative of local subtypes. Subtype designation of reference subtypes was confirmed by sequence analysis of the V3-loop region.

Results: Analysis of the partial *gag* sequences and HMA of the V3–V5 *env* region identified three subtypes: B, C and D. A fourth *env* subtype, subtype E, was also identified by HMA. Subtypes were found to segregate according to mode of transmission, with subtype B viruses found in 96% (25 out of 26) of the male homosexual group and subtype C viruses found in 81% (26 out of 32) of the heterosexual/vertical transmission group. Subtype B viruses were also found in four heterosexual patients, one patient infected by blood transfusion and in two patients with unknown mode of transmission. Subtype D viruses were found in one male homosexual patient and one heterosexual patient. A subtype E virus was identified in a heterosexual patient. No discrepancy was found in subtype designation in samples analysed in both between the *gag* and *env* regions (n = 19).

Conclusions: Subtype B viruses were associated with male homosexual transmission and subtype C viruses with heterosexual transmission, suggesting two independent epidemics. This data may have implications in the selection of appropriate vaccines for different risk groups in the country.

AIDS 1997, **11:**81–87

Keywords: HIV-1 subtypes, South Africa, molecular epidemiology, heteroduplex mobility assay, HIV-1 genetic sequence

[For editorial comment, see pp 113–116]

Introduction

It is estimated that 14 million adults in sub-Saharan Africa are HIV-infected [1], with the major route of transmission being heterosexual contact. In South Africa, unlike the rest of sub-Saharan Africa, HIV-1 was initially spread by homosexual contact [2,3]. The first two South African cases of AIDS, recorded in

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1982, were male homosexuals [4], and until 1987 HIV-1 was diagnosed almost exclusively in men [2,3]. In 1988, an increasing number of HIV-infected women were reported, signalling the start of a second epidemic, and by 1992 the numbers of new cases in women were was roughly equivalent to those in men [2,3]. Results of a 1995 annual antenatal survey of 13741 specimens showed that 10.44% of pregnant women were infected [5]. At this time it was estimated that there were just over 1.8 million HIV-positive persons in South Africa of a total population of over 41 million.

The rapid evolution of HIV-1 is well documented and at least 10 subtypes have been defined in the *env* region [subtypes A–I (M group) and subtype O (O group)] [6,7]. Subtypes A and D are predominant in Central and East Africa [8] and subtype C in Zambia and Malawi [6]. Subtype C was the first HIV genotype from South Africa to be identified [9] and was subsequently shown to be the major virus subtype in the heterosexual population of this country [10]. Subtype B viruses, commonly found in Europe and America, are not often detected in Africa. Investigations into genetic heterogeneity are important in the development and application of vaccines whose efficacy could be influenced by virus variation [11]. It is also possible to use genetic variation to monitor the spread of HIV-1 into new population groups such as in Thailand where distinct subtypes were associated with the independent introduction of HIV-1 into the injecting drug user population (subtype B) and heterosexual population (subtype E) [12].

A preliminary study has been performed investigating subtypes from different risk groups in South Africa [10]. In this study, we investigated the genetic heterogeneity of HIV-1 in 61 individuals presumed to be infected by different modes of transmission. Results showed that distinct subtypes of HIV-1 were associated with the heterosexual and male homosexual populations.

Materials and methods

Patient data

EDTA-treated blood originated from two sources: the HIV/AIDS clinic (Somerset Hospital; ZA001–ZA078; n = 54) and the diagnostic virology laboratory (Groote Schuur Hospital; ZA201–ZA207; n = 7). Somerset Hospital has the major HIV/AIDS adult referral clinic in the Cape Town region and services mainly government hospitals. The clinical and demographic data of the 58 patients infected by heterosexual/vertical transmission and male homo-/bisexual transmission is shown in Table 1. None of the infants listed in Table 1 were born to women in this study. In addition, blood was obtained from a white woman, aged 28 years, infected by a blood transfusion (94ZA008); a mixed-race man, aged 42 years, infected by unknown mode of transmission (94ZA074); and a mixed-race man, age unknown, infected by unknown mode of transmission (94ZA078). Travel history and travel history of sexual partner were recorded.

Polymerase chain reaction, cloning and sequencing

DNA was extracted directly from EDTA-treated blood [13]. A ± 500 base-pair region of the *gag* gene was amplified using outer primers SK28 [14] and SK431 [15], and inner primers LTR236 (5′-CGCAGGACTCGGCTTGC) and *gag*778 (5′-CCCATGC[A/G]TT[T/C]GTTCTAGGTG) which were designed for the study. Forty cycles of amplification were performed using *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany) and recommended buffer conditions. The DNA was denatured at 94°C for 30 sec, primers annealed at 45°C for 30 sec and DNA elongated at 72°C for 60 sec.

A 1.2 kb and 700 base-pair fragment of the *env* gene was amplified in a nested polymerase chain reaction (PCR) using primers ED3/14 and ED5/12 or ED5/12 and ES7/8 [National Institutes for Health (NIH) AIDS Research and Reference Reagent Program, Rockville, Maryland, USA], respectively, as previously described [16]. The 1.2 kb fragment was cloned into pMOS-blue vector (Amersham International, Little Chalfont, Buckinghamshire, UK) for sequencing, whereas the 700 base-pair fragment was used for heteroduplex mobility assay (HMA). For sequencing of 360 bases including the V3 loop, ES7 and ED33 (NIH AIDS Research and Reference Reagent Program) were used as the forward and reverse primers, respectively.

The ± 500 base-pair *gag* fragments (n = 33) and the 1.2 kb *env* fragments (n = 5) were cloned by (i) blunt-end ligation into *Sma*I restriction site of pUC18 (Boehringer) or the *Srf*I restriction site of pCR Script (Stratagene, La Jolla, California, USA); or (ii) by TA, overhang cloning into pMos-blue T-vector (Amersham), as recommended by the manufacturers. Ligated DNA was transformed into *Escherischia coli* and white colonies containing putative clones screened for inserts by *Pst*I/*Eco*RI double digestion (*env* clones) or probing with digoxigenin labelled probe *gag* 1 (5′-GAAGGAGAGAGATGGGTGCG; *gag* clones). DNA from positive clones was purified and both strands were sequenced by standard dideoxy chain termination method using ^{35}S-dATP label.

Table 1. Patient clinical and demographic data by heterosexual/vertical and homo-/bisexual modes of transmission.

Sample	Race	Sex	Age (years)	Year of first serodiagnosis	CD4 count (x10^6/l)
Heterosexual transmission					
93ZA006	White	Male	30	1993	342
93ZA010	Black	Female	41	1993	530
93ZA020	Mixed race	Female	33	1992	5
93ZA023	Black	Male	48	1992	169
93ZA024	Black	Male	41	1993	200
93ZA025	Mixed race	Female	24	1992	375
93ZA029	Black	Female	29	1993	172
93ZA030	Black	Male	58	1991	0
93ZA031	Black	Male	31	1991	154
93ZA032	Black	Female	34	1993	72
93ZA035	Mixed race	Male	52	1993	140
93ZA036	Black	Male	42	1992	625
93ZA037	Mixed race	Male	32	1993	418
93ZA040	Black	Female	34	1990	269
93ZA042	Mixed race	Male	27	1991	54
93ZA043	Mixed race	Female	32	1993	443
93ZA046	Black	Female	21	1993	404
93ZA047	Black	Male	24	1993	373
93ZA048	Black	Male	40	1993	111
93ZA049	Black	Female	20	1992	517
94ZA058	White	Female	37	1991	357
94ZA061	Black	Male	33	1994	138
94ZA063	Mixed race	Male	24	1994	ND
94ZA067	Black	Female	30	1989	ND
94ZA200	Black	Male	Unknown	1994	ND
94ZA201	Mixed race	Female	38	1993	ND
94ZA202	Black	Female	44	1992	ND
94ZA205	Black	Male	21	1992	ND
94ZA206	White	Female	Unknown	1993	ND
Vertical transmission					
94ZA203	Black	Female	0.7	1993	ND
94ZA204	Black	Male	0.1	1993	ND
94ZA207	Black	Unknown	1	1993	ND
Homosexual transmission					
93ZA001	White	Male	37	1988	504
93ZA002	White	Male	35	1991	52
93ZA003	White	Male	32	1990	84
93ZA004	White	Male	30	1984	564
93ZA005	Mixed race	Male	42	1991	231
93ZA007	White	Male	41	1987	0
93ZA009	Mixed race	Male	31	1991	499
93ZA011	Mixed race	Male	25	1991	481
93ZA012	Mixed race	Male	26	1990	550
93ZA017	White	Male	25	1989	8
93ZA019	White	Male	40	1987	534
93ZA021	Mixed race	Male	44	1991	245
93ZA022	White	Male	49	1993	69
93ZA033	White	Male	36	1990	374
93ZA034	White	Male	42	1991	21
93ZA041	Mixed race	Male	30	1993	54
93ZA044	White	Male	48	1991	208
93ZA045	Mixed race	Male	26	1992	397
94ZA050	Mixed race	Male	39	1993	32
94ZA051	White	Male	29	1993	18
94ZA059	Mixed race	Male	55	1994	77
94ZA073	White	Male	47	1989	23
94ZA076	White	Male	Unknown	1994	ND
94ZA077	Mixed race	Male	Unknown	1988	ND
Bisexual transmission					
93ZA038	White	Male	38	1990	0
94ZA072	White	Male	56	1986	45

ND, not determined.

Heteroduplex mobility assay

HMA was performed on the 700 base-pair V3–V5 region of the *env* gene as previously described [16]. For subtype designation, unknown samples were initially compared with reference plasmids A–F in an HMA kit from the NIH AIDS Research and Reference Reagent

Program, after which South African reference plasmids (two B, two C and one D) could be constructed. Subsequent unknown samples were compared with the South African reference plasmids and one subtype A reference plasmid from the NIH AIDS Research and Reference Reagent Program. The heteroduplexes were separated by electrophoresis on a $190 \times 160 \times 1.5 mm^3$ (Hoefer SE600) 5% polyacrylamide gel at 200 V for 5 h. The DNA was visualized by ethidium bromide staining.

Sequence analysis

The sequences were named by year of isolation, country of origin, patient identification number and mode of transmission. Sequences were aligned using CLUSTAL V [17], with a final manual adjustment. Phylogenetic analysis was performed using the distance matrix neighbour-joining method in TREECON for Windows 1.0 [18]. The neighbour-joining trees were constructed using 1000 bootstrap replicates with the simian immunodeficiency virus $SIV_{CPZ\text{-}Gab}$ sequence as outgroup, ignoring insertions/deletions, and using the Kimura 2-parameter correction for multiple substitutions [19]. The *gag* sequence set was also analysed using PAUP 3.1.1 [20] by use of the simple heuristic tree generation algorithm with gaps counted as 'missing' and tree-breaking and resection, with the $SIV_{CPZ\text{-}Gab}$ sequence as outgroup. All the 900 equally parsimonious shortest trees found were used to generate a strict 50% majority rule consensus tree.

Results

Two methods were used to subtype a total of 61 samples from Cape Town: sequence analysis of the p17 region of the *gag* gene, and HMA of the V3–V5 region of the *env* gene.

Phylogenetic analysis of 33 partial *gag* sequences identified three subtypes: B, C and D (Fig. 1a). Nine out of 10 individuals from the male homo-/bisexual group

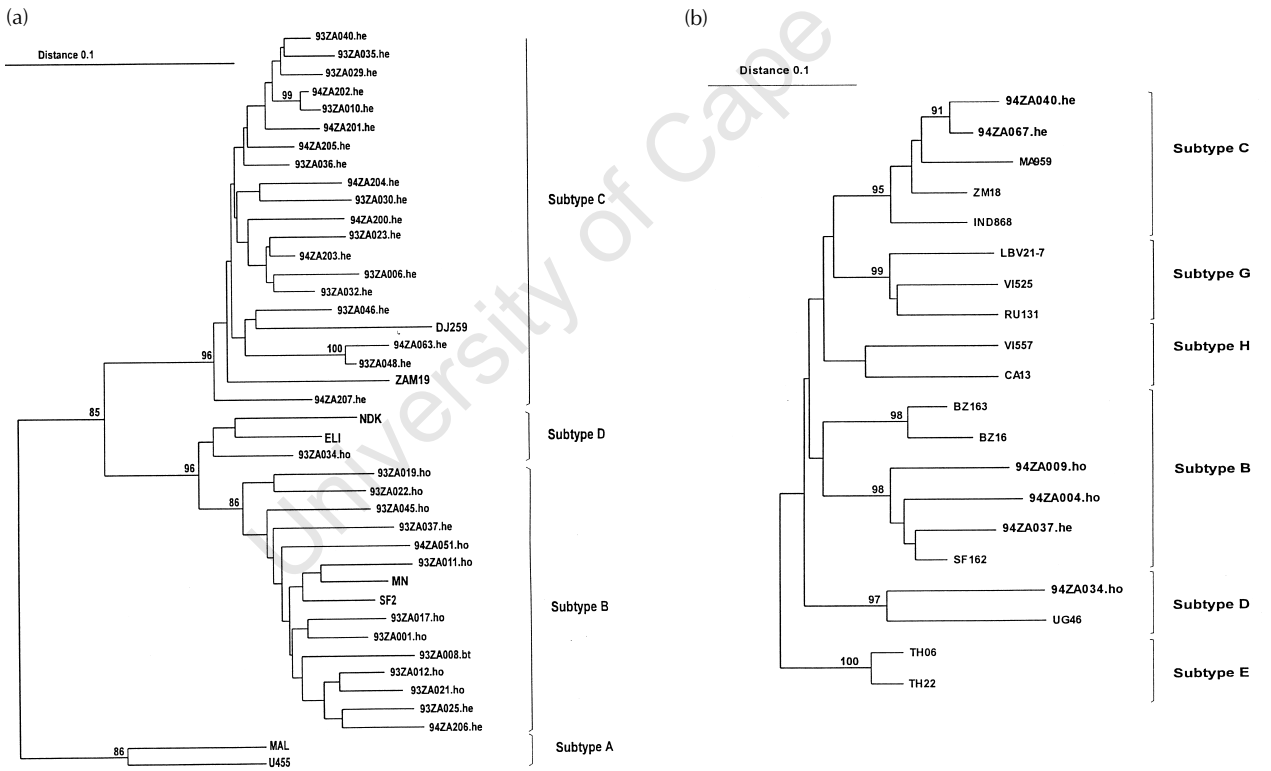

Fig. 1. Neighbour-joining phylogenetic trees resulting from 1000 bootstrap replicates on (a) partial *gag* sequences (441 base-pairs) and (b) V3 region sequences (360 base-pairs). The trees were rooted on the $SIV_{CPZ\text{-}Gab}$ sequence (not shown). Horizontal distances are proportional to sequence distances between species; vertical distances are arbitrary. Numbers along the horizontal branches represent bootstrap scores in percentages: only values of 85% and higher are shown. Samples are named according to year of isolation, country of origin (ZA, South Africa), patient identification number and mode of transmission: ho, homosexual; he, heterosexual; bt, blood transfusion. Country of origin of reference strains are as follows: (a) DJ, Djibouti; ZAM19, Zambia; NDK, Zaire; ELI, Zaire; MN, United States of America (USA); SF2, USA; MAL, Zaïre; U455, Uganda. (b) MA, Malawi; ZM, Zambia; IND, India; LBV, Gabon; RU, Russia; VI, Gabon; CA, Cameroon; BZ, Brazil; SF, Rwanda; UG, Uganda; TH, Thailand.

were infected with subtype B and one with subtype D. Of the 22 people infected by heterosexual/vertical transmission, 19 were infected with subtype C and three with subtype B. The individual infected by blood transfusion was infected with a subtype B virus. The consensus PAUP tree showed very similar topology to the neighbour-joining tree in all major branches, with small differences confined to the terminal branches (not shown). All sequences had intact open reading frames and varied in length from 380 to 420 bases. The average intrasubtype DNA difference for subtypes B and C were 7.6 and 6.7%, respectively, and ranged from 4.6 to 10.9% and from 1.0 to 10.6%, respectively.

Forty-seven samples were subtyped on the basis of the *env* region using HMA. Reference plasmids were constructed containing *env* sequences representative of local strains, as the subtype designation of certain samples was ambiguous using the NIH AIDS Research and Reference Reagent Program reference plasmids. Three subtype B, two subtype C and one subtype D reference plasmids were constructed and their subtype designation confirmed by sequence analysis of 360 bases of the V3-loop region of the *env* gene (Fig. 1b). The tetrapeptide crown of the V3 loop was GPGR in all three of the subtype B viruses. The two subtype C viruses and one subtype D virus had a GPGQ tetrapeptide crown motif. Four different subtypes were identified by HMA: B, C, D and E. Of the 23 patients in the male homo-/bisexual group, 22 were infected with subtype B and one with a subtype D virus. Of the 21 patients in the heterosexual group, 17 were infected with subtype C, two with subtype B, one with a subtype D, and one with a subtype E virus. Three additional subtype B viruses were identified, two transmitted by unknown route and one by blood transfusion.

Nineteen samples subtyped by HMA based on the *env* gene were also subtyped by sequence analysis of the partial *gag* gene. There was no discrepancy in subtype designation among this group. The results of subtyping by sequencing and HMA are summarized in Table 2.

All patients were residing in Cape Town at the time of the study. The following homosexual men reported having had sexual contact with men while travelling in foreign countries in the 5 years prior to sampling: ZA004, ZA005 (United States); ZA003, ZA005, ZA007, ZA019 (Europe); ZA022, ZA033 (Australia); and ZA050 (Thailand). All these individuals were infected with subtype B viruses. Heterosexual men who reported having had sexual contact while in nearby Southern African countries within 5 years of sampling included ZA020, ZA030 (Zambia); ZA024 (Namibia); ZA031 (Zimbabwe); and ZA200 (Malawi). All these individuals were infected with subtype C viruses.

Discussion

There have been two patterns of infection in South Africa on two time scales: in the early 1980s the epidemic affected mainly the male homosexual population and by the late 1980s this had shifted to a predominantly heterosexual epidemic [2,3]. In this study, four subtypes were identified: 32 subtype B, 26 subtype C, two subtype D viruses and one *env* subtype E virus. It would be of interest to sequence the subtype E virus in the *gag* and *env* regions to determine whether the strain is an *env* E–*gag* A recombinant such as has been found in Thailand and the Central African Republic [6]. The subtypes segregated according to mode of transmission with 96% (25 out of 26) of the male homo-/bisexual group infected with subtype B viruses and 81% (26 out of 32) of the heterosexual/vertical group infected with subtype C viruses. There is a significant association between subtype and mode of transmission ($P<0.001$), which suggests two independent epidemics and that the origin of the second epidemic was largely independent of the first wave of infections. Many of the homosexual men in this study reported having had sexual contact while travelling abroad. In addition, a study performed in 1983 on 32 HIV-positive homosexual men reported that the majority reported having had sexual contact with men in the United States or Europe [21]. Thus both epidemiological and molecular data suggest that the initial epidemic in South Africa was, at least in part, a result of the introduction of HIV-1 into South Africa from other continents. In addition, a subtype D virus, commonly found in Central Africa [6], was identified in a male homosexual patient from our study.

Table 2. HIV-1 subtype designation according to presumed mode of transmission.

Region used for subtyping	Heterosexual/vertical				Homo-/bisexual		Unknown	Blood transfusion
	B	C	D	E	B	D	B	B
env	1	7	1	1	16	–	2	–
gag	2	9	–	–	3	–	–	–
env and *gag*	1	10	–	–	6	1	–	1
Total	4	26	1	1	25	1	2	1

The heterosexual epidemic is a relatively recent epidemic, as the first cases in women were reported in the mid-1980s [2,3]. The intrasubtype DNA distance for subtype C viruses was greater than expected for a clonal HIV-1 epidemic and it is probable that there was multiple introduction of the same subtype into the heterosexual community. This is supported by epidemiological data whereby some of the patients in this study reported having had sexual contact in Zambia, Malawi and Zimbabwe, where this subtype is found [6,22]. The origin of this second epidemic is therefore probably due to regional spread. Since there are close socioeconomic ties between South Africa and its neighbouring countries, there is a constant traffic of people facilitating the spread of HIV-1 to South Africa.

The global spread of subtypes is a dynamic process and the association between subtype and mode of transmission has been observed in several regions where there are relatively new epidemics including Thailand, Russia, China, and now South Africa [10,12,23,24]. It is possible that certain subtypes have different phenotypic properties resulting in a selective advantage in alternative routes of transmission. A recent study in Thailand showed that subtype E viruses may be associated with higher risk of heterosexual transmission than subtype B viruses [25]. Langerhans' cells are thought to be the primary target cells in heterosexual transmission and it was shown that subtype E, and probably C, replicate more efficiently in these cells than subtype B [26]. In the last few years there has been rapid spread of subtype C viruses throughout the world [23,24,27,28] and this subtype may have a selective advantage in heterosexual transmission. Subtype D viruses were previously reported in five out of 11 South African male homosexual patients diagnosed in the early to mid-1980s [29]. However, in our own study, out of 26 male homosexual patients diagnosed in the late-1980s to early-1990s only one was infected with a subtype D virus. Further work needs to be done to determine whether virus genotype affects the transmissibility and pathogenicity of HIV-1, and studies to further explore this may be possible in South Africa.

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Independent epidemics of heterosexual and homosexual HIV infection in South Africa—survival differences

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Summary

Survival with HIV infection is shorter in sub-Saharan Africa than in developed countries. The pattern of HIV transmission in our region has changed from homosexual to heterosexual, with viral subtypes similar to those in North America/Europe and Central Africa, respectively. We compared survival for the two transmission patterns after AIDS, and after the first CD4+ lymphocyte counts <200/μl and <50/μl, for adults presenting 1988–1993. Antiretroviral therapy was excluded. There were 180 homosexuals (63% White, 56% employed) and 314 heterosexuals (67% Black, 34% employed). Extrapulmonary tuberculosis was the AIDS-defining diagnosis in 36/90 heterosexuals and 5/58 homosexuals ($p<0.0001$). Survival after AIDS was longer in heterosexuals ($p=0.0015$), but AIDS occurred earlier as shown by their higher CD4+ count at AIDS onset (median 98/μl vs. 40/μl; $p=0.036$). Survival was similar in the two groups after first CD4+ count <200/μl and <50/μl. Race, socioeconomic status and morbidity are markedly different in the two transmission groups. AIDS occurs with less severe immune suppression in heterosexuals, with correspondingly longer survival. Survival after defined CD4+ counts, however, is remarkably similar.

Introduction

The prognosis of HIV infection in sub-Saharan Africa has been reported to be worse than in industrialized countries.[1,2] This may be due to death from endemic non-opportunistic infections (particularly *Mycobacterium tuberculosis*, the salmonellae, and *Streptococcus pneumoniae*) prior to the onset of severe immunosuppression.[1] This seems plausible given the overburdened and poorly-funded health-care facilities in most countries in this region. Death prior to the onset of severe immunosuppression may also explain the observed scarcity of many opportunistic infections in HIV-infected patients in Africa.[1,3]

Alternatively, the poorer prognosis in Africa may be due to more rapid disease progression as a result of more virulent HIV subtypes, genetic predisposition, or the presence of co-factors. Supporting this hypothesis, the Nairobi sex-worker cohort study reported progression to AIDS twice as rapid as that in cohorts from industrialized countries.[4]

The transmission pattern of HIV in our area has changed from an initial homosexual/bisexual epidemic (pattern 1) to a heterosexual epidemic (pattern 2). Our pattern 1 patients have viral subtype B (the same as in North America/Europe), whilst pattern 2 patients have subtype C, which occurs in sub-Saharan Africa,[5] indicating that the two epidemics are independent. Health-care facilities in South Africa are better than elsewhere in sub-Saharan Africa,[6] which enables us to diagnose and treat most opportunistic infections, and monitor serial CD4+ lymphocyte counts. However, anti-retroviral therapy is not generally available in the state health sector. We are thus in a unique position to compare the outcome of the two transmission patterns in an HIV-infected population unexposed to anti-retroviral therapy.

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Methods

Somerset and Groote Schuur hospital HIV clinics are both affiliated to the University of Cape Town. The same treatment protocol is used at both clinics, and medical personnel are partly shared. Patients were referred from primary care, often following screening for insurance or blood donation, or from hospitals in the Western Cape region. There was no private specialist HIV care available. Adult patients attending for their initial visits between 1988–1993 were studied. All patients had HIV-1 infection confirmed by ELISA and Western blot or three different ELISAs on specimens drawn on two occasions. Data were acquired by retrospective chart review prior to December 1991, and prospectively thereafter. Follow-up continued until April 1995. CD4+ lymphocyte counts were performed 3- to 6-monthly by flow cytometry. Demographic data, including sexual preferences and employment status, were collected. Patients who were taking anti-retroviral therapy were excluded. Cotrimoxazole primary prophylaxis was routinely prescribed from 1991 for patients with CD4+ counts $<200/\mu l$. Primary prophylaxis for fungal or mycobacterial infections was not used.

Date of death was obtained from hospital records or from deaths reported to the clinics by relatives or friends. In addition, regional death records were searched if patients failed to attend for more than 6 months.

Statistical analysis was done using the software packages EpiInfo version 6 (Centers for Disease Control), SAS and Statgraphics version 6 (Statistical Graphics). Survival from the first AIDS diagnosis (1987 Centers for Disease Control case definition)[7] and the first recorded CD4+ lymphocyte counts $<200/\mu l$ and $<50/\mu l$ were assessed by Kaplan-Meier analysis. Patients were right censored if their last clinic attendance was before 2 years follow-up. The survival curves for patterns 1 and 2 were compared using log rank tests. Yates' correction was applied to χ^2 tests.

Results

The transmission pattern changed from predominant homosexual transmission to heterosexual transmission during the study period (Figure 1). There were 662 patients whose first clinic visit was between 1988 and 1993. Anti-retroviral therapy was taken at some stage by 112 patients, and sexual preference was not recorded in 56, leaving 494 evaluable patients (36% pattern 1, 64% pattern 2). Demographic data is shown in Table 1. Pattern 2 patients were significantly younger ($p<0.01$). There were no intravenous drug users. Employment status was assessed only for males. Because being unemployed at the time of presentation may reflect disability due to HIV, we also evaluated prior employment status. The proportion of males who had ever worked was 70.6% in pattern 1 compared with 47.5% in pattern 2 ($p<0.0001$). In those patients who had ever worked, unskilled or semi-skilled labour was performed by 18% of pattern 1 patients compared with 73.5% of pattern 2 patients ($p<0.0001$).

Nine patients in each transmission pattern died prior to the onset of AIDS ($p=0.308$). The degree of immunosuppression in each group was similar as assessed by CD4+ lymphocyte count at first visit (357 ± 244 pattern 1; 312 ± 221 pattern 2; $p>0.05$) and the proportion with AIDS at first visit (13% pattern 1; 17% pattern 2; $p=0.18$). The mean time to developing AIDS was also similar (12.3 months pattern 1; 11.3 months pattern 2; $p>0.05$). Medical care was similar, as assessed by the number of clinic visits per year prior to developing AIDS (6.3 visits pattern 1; 5.5 visits pattern 2; $p>0.05$)

AIDS was diagnosed on entry or subsequently occurred in 148 patients (58 pattern 1, 90 pattern 2). The commonest AIDS-defining conditions are listed in Table 2. *Pneumocystis carinii* pneumonia

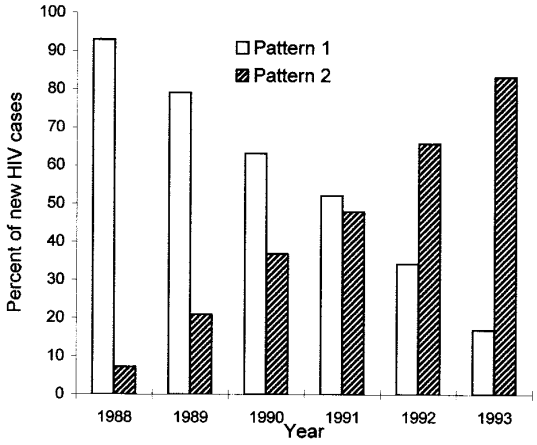


Figure 1. The proportion of new pattern 1 and 2 patients attending the HIV clinics in Cape Town from 1988 to 1993.

Table 1 Demographic data

	Pattern 1	Pattern 2
Race		
White	114 (63.3%)	11 (3.5%)
'Mixed'	65 (36.1%)	93 (29.5%)
Black	1 (0.6%)	210 (67%)
Age (years)	33.1 ± 8.95	30.22 ± 8.94
Males	180 (100%)	147 (46.8%)
Employed*	101 (56.1%)	50 (34%)

*Employment status applies only to males.

Table 2 Most frequent AIDS-defining diagnoses in the two transmission patterns

AIDS-defining condition	Pattern 2 (n = 90)	Pattern 1 (n = 58)	Odds ratio (95% CI)
Extrapulmonary TB	36 (40%)	5 (9%)	7.07 (2.47–24.56)
Pneumocystis carinii	6 (7%)	11 (19%)	0.31 (0.09–0.98)
Kaposi's sarcoma	8 (9%)	8 (14%)	0.61 (0.19–2.0)
Candidosis	10 (11%)	5 (9%)	1.33 (0.39–5.22)
Herpes simplex	7 (8%)	6 (10%)	0.73 (0.2–2.8)
Wasting syndrome	5 (6%)	7 (12%)	0.43 (0.1–1.67)

TB, tuberculosis.

occurred more frequently in pattern 1 patients ($p = 0.043$) whilst extrapulmonary tuberculosis occurred more frequently in pattern 2 patients ($p < 0.0001$). CD4+ lymphocyte counts at the time of AIDS-defining diagnosis were available in 53 patients. These were lower in pattern 1 patients (median 40/μl; interquartile range 16–126) compared with pattern 2 patients (median 98/μl; interquartile range 47–194; $p = 0.036$, Mann-Whitney U test). Pattern 2 patients survived significantly longer ($p = 0.0015$) following AIDS diagnosis (evaluable in 130 patients)(Figure 2).

Survival from the first recorded CD4+ lymphocyte count <200/μl ($n = 190$) and <50/μl ($n = 82$) was similar for both patterns (Figure 3). Survival from the first CD4+ lymphocyte count <200/μl was similar for male and female pattern 2 patients ($p = 0.33$). Controlling for age by Cox proportional hazard in males with CD4+ lymphocyte count <200/μl showed no significant difference in survival (risk ratio for death in pattern 1 patients 1.857, 95% CI 0.975–3.537; $p = 0.06$). In this group, survival was shorter for pattern 1 compared with male pattern 2 patients <33 years ($p = 0.018$) but similar for males 33 years or older ($p = 0.55$) (a cutpoint of 33 years was chosen because it gave approximately equal numbers in the sexual preference groups). We also controlled for the time when AIDS or the first CD4+ count <200/μl or <50/μl was found, as the quality of care may have changed over time. This did not alter the findings that pattern 2 patients survived longer after AIDS and that survival was similar after first CD4+ count <200/μl or <50/μl.

Discussion

The major finding of our study was that survival, when assessed from CD4+ counts <200/μl and <50/μl, was similar for both major transmission

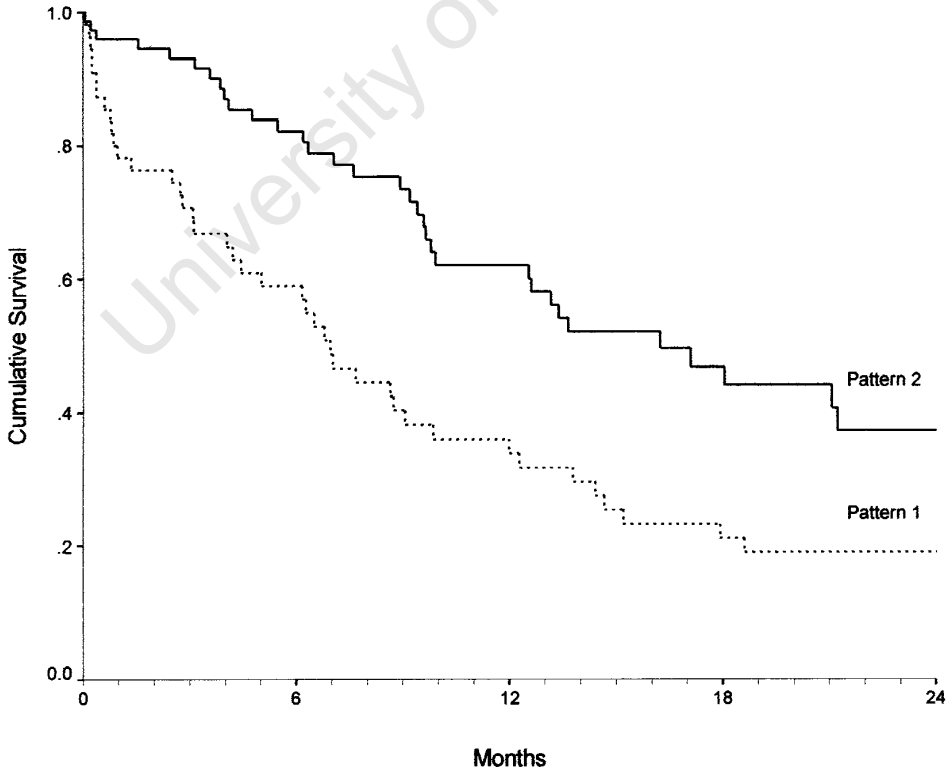
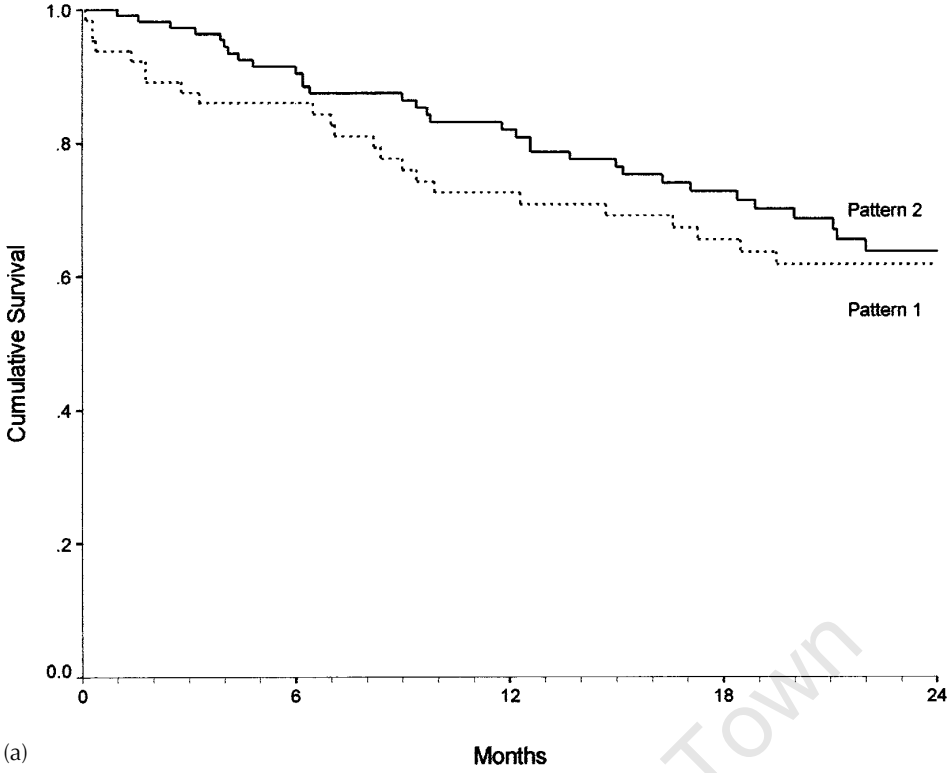
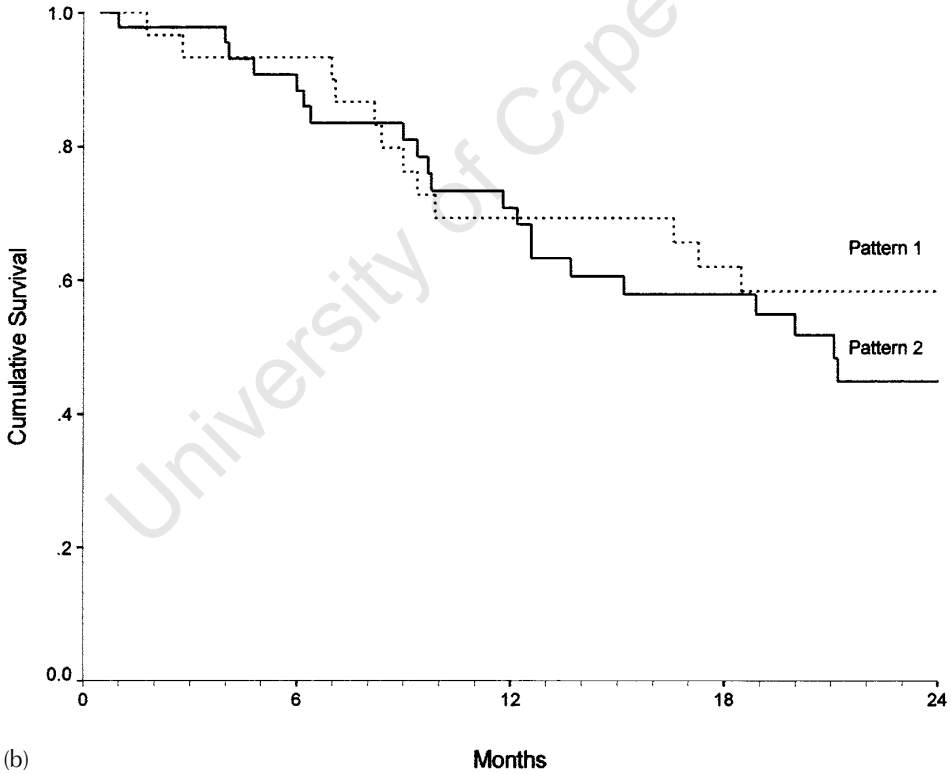


Figure 2. Cumulative survival after the first AIDS diagnosis. Survival was longer in pattern 2 patients ($p = 0.0015$).



(a)



(b)

Figure 3. Cumulative survival following CD4+ lymphocyte counts **a** <200/μl and **b** <50/μl. Survival was similar for pattern 1 and 2 patients (**a** $p=0.206$; **b** $p=0.91$).

patterns. This was not due to age differences, which are important prognostically,[8] between the two groups. Although our observations were largely confined to late HIV disease, the fact that there were no excess deaths in pattern 2 patients before AIDS provides further support to our finding that disease

progression is similar for both transmission patterns. The longer survival of pattern 2 AIDS patients is reflected in their higher frequency of extrapulmonary tuberculosis. This presents with higher CD4+ lymphocyte counts than most other AIDS-defining conditions, and is associated with longer survival.[9]

The CDCs expanded definition of AIDS[10] has been criticized by one centre, which found that the revised definition led to a threefold increase in patients defined as having AIDS, and their survival was more than twice as long.[11] The use of a CD4+ lymphocyte $<200/\mu l$ to define AIDS is problematic, because of considerable differences in results from different laboratories.[12] These problems apply to industrialized countries—resource-poor countries are generally unable to afford CD4+ lymphocyte determinations. In addition, the inclusion of pulmonary tuberculosis as an AIDS-defining condition is not applicable to areas where tuberculosis is endemic.[13] Therefore we used the 1987 CDC case definition of AIDS.[7]

In sub-Saharan Africa, the reason for the scarcity of certain major opportunistic infections is thought to be due to death before the development of severe immunosuppression.[1,3] In our pattern 2 patients, tuberculosis continued to predominate, even with severe immunosuppression. Tuberculosis is endemic in the African and mixed race communities in our area.[14] We do see the major opportunistic infections in our pattern 2 patients, with the notable exception of *Mycobacterium avium* complex infection, although this occurs in our environment.[15] Geographical differences in the incidence of these infections may be due to the protective effect of BCG vaccination,[16,17] which the majority of patients of all races in Cape Town have received.[14]

Shorter survival has been reported for HIV-infected patients with lower socioeconomic circumstances.[18] Socioeconomic status, as assessed by current or previous employment status, was markedly lower in our pattern 2 patients. Despite this, their survival was similar to pattern 1 patients. The likely explanation for this is that patients were treated with the same quality of care. This view is supported by a recent American study from a single centre which also found that socioeconomic status was not a determinant of outcome.[19]

Our pattern 2 patients are similar to those elsewhere in sub-Saharan Africa in terms of their morbidity (predominantly tuberculosis and non-opportunistic bacterial infections[20]) and their HIV-1 viral subtype.[5] The shorter survival in HIV-infected patients observed elsewhere in Africa is thought to be largely due to death from non-opportunistic infections because of poor access to inadequate health care.[1] The fact that survival is similar for both transmission patterns in Cape Town is presumably due to our better health-care resources,[6] which enables us to investigate and treat most HIV-related infections. This is illustrated by the similar survival of our AIDS patients to those in Europe[9] and New York.[21] Our patients with severe immune suppression (a CD4+ lymphocyte count $<50/\mu l$) have a median survival similar to that reported from the USA.[22] It is noteworthy that this similar survival in late HIV disease was achieved without anti-retroviral therapy.

Acknowledgements

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Appendix

Life tables of survival analysis—numbers refer to evaluable patients or the number who died.

Appendix to Figure 2—survival after AIDS

	0 months	6 months	12 months	18 months	24 months	Deaths
Pattern 1	55	30	17	10	7	41
Pattern 2	75	50	31	17	8	35

Appendix to Figure 3a—survival after CD4 < 200

	0 months	6 months	12 months	18 months	24 months	Deaths
Pattern 1	67	54	42	36	31	23
Pattern 2	123	91	74	55	37	32

Appendix to Figure 3b—survival after CD4 < 50

	0 months	6 months	12 months	18 months	24 months	Deaths
Pattern 1	33	29	20	16	15	13
Pattern 2	49	39	29	20	12	22

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Disseminated histoplasmosis in AIDS patients in South Africa: a report of three cases and review

R Wood, T Jackson

Histoplasmosis has not been previously reported in South African AIDS patients. Three cases of disseminated* Histoplasma capsulatum *infection are described in HIV-infected individuals living in Cape Town who did not belong to previously recognised risk groups for exposure to* H. capsulatum. *The clinical presentations were predominated by systemic symptoms with the diagnosis being confirmed by tissue histology and fungal culture. Histoplasmosis is amenable to treatment, associated with prolonged survival and should be included in the differential diagnosis of HIV-infected patients in South Africa with fever, wasting, hepatomegaly, pulmonary infiltrates or mucocutaneous lesions.

Introduction

Histoplasma capsulatum infection in immune competent individuals typically causes a mild primary pulmonary infection which rarely disseminates.[1] In South Africa *H. capsulatum* infection has been considered a rare disease, affecting predominantly spelaeologists exposed to bat infested caves[2-5] with occasional case reports of disseminated disease in other groups.[6-9] In recognised endemic areas of the USA, the immune suppression associated with HIV infection increases the risk of dissemination, resulting in *H. capsulatum* infection in up to 27% of AIDS patients.[10] In contrast, disseminated *H. capsulatum* infection in AIDS has been very rarely reported in African patients[11-14] and not previously reported in South Africa. We describe three cases of disseminated histoplasmosis occurring in HIV-infected individuals living in Cape Town.

Patients and methods

Case 1

A 39-year-old white male presented with a history of fever and weight loss. Four months prior to admission he had been treated for an acute respiratory illness, while working in a recently reopened underground diamond mine in the Free State. Following this illness he lost approximately 30 kilograms of weight and noted increasing dyspnoea on effort. On examination the patient was wasted, dyspnoeic at rest with a temperature of 40°C and pulse rate of 110/min. A diffuse papular erythematous rash involving the face, trunk and limbs was present but there was no significant lymphadenopathy and no mouth lesions. Examination of the cardiovascular system and abdomen was normal. Auscultation of the chest revealed normal vesicular breath sounds. Central nervous system examination was completely normal.

Results of laboratory investigations were: haemoglobin 9.2 g/dl, leucocyte count 2.88×10^9/L, neutrophils 98%, lymphocytes 2%, CD4 count 8×10^6/L, CD8 count 23×10^6/L, sodium 125 mmol/L, potassium 4.0 mmol/L, urea 3.4 mmol/L, creatinine 45 µmol/L, AST 125 IU/L, ALP 280 IU/L, bilirubin 11 µmol/L, HIV-1 serology positive and serum cryptococcal antigen negative.

Chest radiograph showed a diffuse interstitial infiltrate involving both lung fields. A skin biopsy demonstrated a perivascular neutrophil infiltrate with narrow-budding encapsulated yeasts compatible with *H. capsulatum* seen lying free within the dermis and within macrophages (Grocott silver stain). Mucicarmine staining for *Cryptococcus neoformans* polysaccharide was negative. Treatment was commenced with oral fluconazole (200 mg/day) with rapid resolution of the rash and respiratory symptoms. Two months after starting therapy, the patient was no longer dyspnoeic, the chest radiograph infiltrates had cleared and his weight had increased by 20 kilograms. The patient remained well and in full employment on maintenance fluconazole (100 mg/day) at 12 months' follow-up.

Case 2

A 30-year-old HIV positive white male chef presented with a respiratory illness characterised by fever, non productive cough, arterial oxygen desaturation on exercise and a normal chest radiograph. The patient had not travelled outside the Western Cape and gave no history of exposure to caves, mines or bat guano. A presumptive diagnosis of *Pneumocystis carinii* pneumonia was made and treatment commenced with high dose cotrimoxazole with rapid resolution of respiratory symptoms. A generalised erythematous papular rash developed 21 days later. On examination the patient was not dyspnoeic, temperature was 39°C and pulse rate was 105/min. A diffuse papular erythematous rash involving the face, trunk and limbs was present but there was no significant lymphadenopathy and no mucocutaneous ulceration. Examination of the abdomen revealed moderate hepatosplenomegaly. Respiratory and nervous system examination was normal.

Results of laboratory investigations were: haemoglobin 9.6 g/dL, leucocyte count 4.93×10^9L, neutrophils 53%, lymphocytes 31%, monocytes 13% and atypical lymphocytes 2.6%, CD4 count 12×10^6L, CD8 count 1283×10^6L, sodium 132 mmol/L, potassium 3.9 mmol/L, urea 3.0 mmol/L, creatinine 66 µmol/L, AST 66 IU/L, ALP 49 IU/L, bilirubin 10 µmol/L, HIV-1 serology positive and serum cryptococcal antigen negative.

Chest radiograph was normal. A skin biopsy demonstrated small encapsulated budding yeasts, and culture of biopsy tissue grew the dimorphic fungus *H. capsulatum*. Treatment was started with oral fluconazole (200 mg/day) but new skin lesions continued to develop. Treatment was changed to intravenous amphotericin B

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which was continued for six weeks with eventual resolution of skin lesions and maintenance therapy with itraconazole (100 mg/day) was continued. The patient returned to full time employment and was asymptomatic at three months' follow-up.

Case 3

A 52-year-old HIV positive black male screen printer presented with a four-month history of weight loss, non productive cough and decreasing effort tolerance. He had been born in the Eastern Cape and lived most of his life in Cape Town, except for a short residence in Gabarone, Botswana. There had been no exposure to mines or caves. On examination he was pyrexial at 40.2°C, mildly obtunded with oral candidiasis and shotty lymphadenopathy. Sparse pigmentation papules were present on the limbs but no oral ulceration. Respiratory, cardiac and abdominal systems were normal.

Result of laboratory investigations were: HIV-1 serology positive, haemoglobin 12.2 g/dL, leucocyte count $2.2x10^9$/L, neutrophils 77%, lymphocytes 12%, eosinophils 2%, platelets $103x10^9$/L, CD4 count $2x10^6$/L, CD8 count $249x10^6$/L, sodium 135 mmol/L, potassium 5.3 mmol/L, urea 5.0 mmol/L, creatinine 124 µmol/L, AST 200 IU/L, ALP 64 IU/L, bilirubin 12 µmol/L and INR 1.0; cerebral spinal fluid analysis: protein 4.4, glucose 3.3, red cells 3/hpf, lymphocytes 4/hpf, Gram stain negative, fungal culture negative, cryptococcal antigen negative; serum cryptococcal antigen negative and histoplasma precipitin test negative.

Chest radiograph was normal. A liver biopsy showed portal tract histiocytes containing numerous intracellular budding yeasts. Fungal culture of hepatic tissue grew *H. capsulatum*. Pyrexia settled rapidly after starting intravenous amphotericin B and subsequent maintenance therapy with fluconazole (100 mg/day).

Discussion

The high rate of dissemination of histoplasmosis in HIV-infected individuals has resulted in increased recognition of this infection in areas where it was not previously thought to be endemic. In West Africa, a large HIV post-mortem study demonstrated *H. capsulatum* infection in 2% of autopsies[13] and disseminated histoplasmosis was diagnosed in three of 100 AIDS patients who had been long term residents in Central Africa.[11] HIV-associated histoplasmosis reported in non-endemic areas may result from reactivation of latent disease in individuals with prior travel to endemic regions,[16] or from new acquisition of disease in areas not previously recognised as endemic for histoplasmosis.[17,18] In the 1950s a limited histoplasmin survey performed in South Africa identified cutaneous reactivity, in fungal but not general laboratory workers and in individuals exposed to caves in the Transvaal but not in the Cape Province.[2] More recently, a single cave in the Cape Province has been recognised as the source of three outbreaks of acute pulmonary infection in cavers.[4,5] The full extent of environmental exposure in South Africa, however, has not been assessed because population-based histoplasmin surveys have never been performed, acute histoplasmosis is frequently unrecognised and serological tests lack sensitivity and specificity for reliable diagnosis or seroprevalence studies. While the source of infection can not be established with certainty in Case 1, circumstantial evidence points to occupational underground exposure. The mine workings had been disused for a prolonged period during which they may have become contaminated with bat guano, a recognised source of histoplasmosis infection in caves of the region.[2] An initial pneumonic illness three weeks after starting work in a previously disused mine is consistent with acute pulmonary *H. capsulatum*. The subsequent weight loss, fever, skin rash and chest radiological findings are typical of patients with AIDS and acute disseminated histoplasmosis.[19] Neither of the other two patients had travelled to an endemic area or reported exposure to underground caves, fungal laboratories, bats or bird guano. The source of *H. capsulatum* in these cases is unknown but as neither belonged to previously recognised risk groups, this may indicate that environmental exposure to this organism is more widespread than previously recognised.

The predominance of systemic symptoms seen in all three cases is similar to the clinical findings of a series of 70 histoplasmosis patients, in which fever was present in 100%, significant weight loss in 84% and anaemia in 72%.[20] The clinical features of histoplasmosis in AIDS patients are protean and disease may involve many organs including the liver, spleen, lungs, skin or gastrointestinal tract.[10-22] Infection almost invariably occurs in HIV-infected individuals with very low CD4 T-cell counts.[15] A fungal aetiology was sought in our patients, because of severe immune suppression and systemic symptoms, together with a prominent papular skin eruption in Cases 1 and 2 and hepatomegaly in Case 3. The diagnosis can be established by demonstration of the organism in culture, on direct microscopy of bone marrow or by demonstration of the yeast forms in tissue sections. The tissue forms of *H. capsulatum* are narrow-based encapsulated budding yeasts, 1-5 µ in size, which can be seen both free in the extracellular tissues and intracellularly within macrophages. Other fungi such as *Blastomyces dermatitidis, Torulopsis glabrata* and *Penicillium marneffei* may rarely mimic the extracellular tissue forms of *H. capsulatum*. The usefulness of biopsy has been demonstrated in a study of Brazilian AIDS patients with histoplasmosis, in whom the histology of mucocutaneous lesions led to confirmation of diagnosis in 68% of patients.[21] In contrast, failure to biopsy skin lesions, presumed to be a drug-related eruption, resulted in the misdiagnosis and death of a patient with AIDS and disseminated histoplasmosis.[22]

Continuous life-long suppressive antifungal therapy is required for AIDS patients with disseminated histoplasmosis, because of a high relapse rate.[10] Favourable initial clinical responses have been reported in 85% of AIDS patients treated with amphotericin B, but relapses occur in up to 20% of those receiving maintenance therapy with this drug.[23] Satisfactory initial responses have also been reported using oral triazole therapy with intraconazole and fluconazole[24] and treatment with amphotericin B is now reserved for life-threatening histoplasmosis. Itraconazole has been the most frequently used triazole, although both itraconazole and fluconazole are effective for maintenance therapy and have been associated with prolonged survival.[25,26]

Histoplasmosis should be included in the differential diagnosis of HIV-infected patients in South Africa with severe immune suppression and fever, wasting, hepatomegaly, pulmonary infiltrates or mucocutaneous lesions.

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LETTERS to the EDITOR

Readers' letters concerning articles in the Journal and letters in its field of interest are invited and will be forwarded to the Editor for consideration for publication.

Please post to: Prof HJ Koornhof, The Southern African Journal of Epidemiology and Infection, PO Box 1038, Johannesburg 2000

Toxoplasma gondii infection in HIV-infected individuals in South Africa

In a review of opportunistic infections in Africa, Jentsch stated that no studies have addressed toxoplasmosis in HIV-infected individuals in South Africa.[1] Clinical data, however, describing this and other opportunistic infections in Cape Town AIDS patients have recently been published in this journal.[2] Toxoplasmic encephalitis was reported in 3% of all AIDS patients in the Cape Town series and was the cause of 12% of neurological AIDS presentations. Toxoplasmosis was associated with advanced immune suppression, as manifested by a mean CD4 T-cell count of 86 x 10^6/L at time of diagnosis and therefore was less frequently the initial AIDS defining condition. Preliminary data describing toxoplasma sero-status of South African HIV-infected patients have also been presented locally.[3] The serological status of individuals attending Somerset Hospital HIV clinic, Cape Town, has been collected prospectively since 1994 and is summarised in Table 1. Toxoplasma sero-testing using the Toxostat™ ELISA assay for IgG (graded 1+ to 3+) and the Toxocap-M™ capture assay

Table 1: ***Toxoplasma gondii* serostatus of 480 consecutive HIV-infected individuals presenting to Somerset Hospital HIV clinic, Cape Town. The seroprevalence for each of the main population groups of the Western Cape is shown together with 95% confidence intervals. Toxoplasma IgG seropositivity was significantly higher in black patients ($p < 0.01$) compared to other population groups.**

HIV patients	Black	Coloured	White
Number tested	327	91	61
IgG positive	140	23	9
IgG positive %	43%	26%	15%
95% conf. intervals	37-48	17-35	6-24

for IgM was performed as per manufacturer's instructions (BioWhittaker Inc. Walkersville, MD, USA) by the South African Institute for Medical Research. No sera testing positive for IgM was confirmed by subsequent testing with an indirect immunofluorescent assay. Positive IgG serology was present in 36% of the total population and was significantly higher in black patients ($p < 0.01$) compared to the other racial groups. There was no significant differences of prevalence between males and females. Forty one percent of all positive IgG sera were reported at high titre (3+). These data indicate that prior exposure to *Toxoplasma gondii* is commonest in the South African population group in which HIV infection is most prevalent.[4] It has been estimated that approximately 30% of African AIDS patients who are seropositive for toxoplasma will ultimately develop toxoplasmic encephalitis[5] with the risk being highest in those patients with high titre.[6] The incidence of cerebral toxoplasmosis may therefore be expected to increase, as the South African HIV epidemic matures and an increasing proportion of patients have advanced immune suppression Clinical experience in the developed world has shown that the combination of multiple intracerebral ring enhancing lesions on computer tomography and a positive IgG toxoplasma serology has an 80% positive predictive value for the diagnosis of cerebral toxoplasmosis.[7] The main differential diagnosis for multiple ring enhancing lesions in the USA is multifocal lymphoma, whereas in South Africa, cerebral tuberculomata must also be considered. The specificity of such a clinical algorithm for focal neurological lesions applied in South Africa may, however, be lower due to the high prevalence of both toxoplasmosis and tuberculosis. The high toxoplasma seroprevalence in black HIV-infected patients also reinforces the need for co-trimoxazole prophylaxis, despite the lower incidence of pneumocystis carinii penumonia in this population,[2] as this antimicrobial gives protection against toxoplasmic encephalitis.[8]

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Human Immunodeficiency Virus-Related Abdominal Pain in South Africa

Aetiology, Diagnosis, and Survival

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O'Keefe EA, Wood R, Van Zyl A, Cariem AK. Human immunodeficiency virus-related abdominal pain in South Africa. Aetiology, diagnosis, and survival. Scand J Gastroenterol 1998;33:212–217.

Background: Abdominal pain in acquired immunodeficiency syndrome (AIDS) patients is often a marker of an underlying opportunistic pathologic condition. There are no data on HIV-related abdominal pain in Africa. *Methods:* Forty-four consecutive Cape Town patients with advanced human immunodeficiency virus (HIV) infection (CD4 < 200) and abdominal pain were studied prospectively to determine aetiology and survival. *Results:* A probable cause of pain was identified in 37 (84%): disseminated *Mycobacterium tuberculosis* infection in 11, cryptosporidiosis in 6, cytomegalovirus infection in 6, and atypical mycobacterial infection in 2. Gastrointestinal lymphoma and pancreatitis were not seen. Fever, hepatomegaly, respiratory symptoms, abnormal chest radiograph, and adenopathy, ascites, or abscesses on ultrasound had predictive diagnostic value for disseminated *M. tuberculosis*. Fifty-one per cent of abdominal pain patients survived 6 months, compared with 73% of all AIDS patients ($P < 0.001$). *Conclusions:* The aetiology of HIV-related abdominal pain in Cape Town reflects the high local prevalence of tuberculosis. Clinical and ultrasound features facilitate diagnosis. Abdominal pain is associated with poor survival.

Key words: Abdominal pain; abdominal ultrasound; acquired immunodeficiency syndrome; Africa; atypical mycobacterial infection; cryptosporidiosis; cytomegalovirus; human immunodeficiency virus; survival; tuberculosis

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Gastrointestinal symptoms occur in up to 60% of acquired immunodeficiency syndrome (AIDS) patients in developed countries and 90% in Africa (1). Abdominal pain is a common problem at all stages of human immunodeficiency virus (HIV) disease; previous retrospective series in industrialized countries have documented a prevalence of between 12% and 15% in AIDS patients (2, 3), whereas 45% of AIDS outpatients in Cape Town, South Africa, reported having had abdominal pain in the preceding month (4).

There are few data on the aetiology of HIV-related abdominal pain in industrialized countries and none from Africa. Abdominal pain in early HIV disease may have an aetiology to similar that in HIV-seronegative persons, whereas in advanced disease it is often a marker of underlying opportunistic infection or malignancy. Gastrointestinal lymphoma, cytomegalovirus (CMV) colitis and *Mycobacterium avium intracellulare* (MAI) enteritis were the most commonly implicated causes of abdominal pain in Italian AIDS patients (2). Thuluvath et al. (5) found that the predominant site of pain had predictive diagnostic value, with the commonest cause of right upper quadrant pain being sclerosing cholangitis and diarrhoea frequently being associated with more diffuse abdominal pain.

Tuberculosis is highly prevalent in Africa and particularly in the Western Cape region of South Africa, where the incidence is over 700 per 100,000 (6). In Cape Town, as in Central Africa, extrapulmonary tuberculosis is the commonest AIDS-defining illness, and atypical mycobacterial and CMV infections have been reported less commonly than in developed countries (7). The aetiology of abdominal pain in South African AIDS patients might be expected to reflect this different spectrum of opportunistic conditions.

We set out to determine prospectively the prevalence of conditions associated with abdominal pain in a multiracial group of patients with advanced HIV disease (CD4 < 200) in Cape Town, South Africa, and to determine whether an underlying diagnosis of disseminated *M. tuberculosis* infection could be predicted by simple measures such as clinical presentation, chest radiograph appearance, and abdominal ultrasound findings. The prognostic significance of abdominal pain in advanced HIV infection was studied by comparing survival of this patient cohort with that of AIDS patients from the same population.

SUBJECTS AND METHODS

Setting

Somerset Hospital, Cape Town, is a well-established referral centre for HIV-infected patients from the Western Cape region of South Africa. The population served is multi-

racial (white, black, and mixed race), and subjects at all clinical stages of HIV disease are seen. The clinic is staffed by two full-time physicians (E. O'Keefe, R. Wood), who are also responsible for HIV inpatient care. Outpatient visit data have been recorded on a computer database (Epi Info 6.02) since 1984 and are updated weekly.

Subjects

HIV-infected subjects (in- and out-patients) presenting to Somerset Hospital with new onset of abdominal pain 1 week or more in duration and a CD4 count < 200 were studied prospectively from November 1994 to September 1996. Most of these patients were taking prophylaxis against *Pneumocystis carinii* pneumonia, but antituberculous prophylaxis was not routinely prescribed. Antiretroviral therapy was not available within the state health system.

The site of abdominal pain, the presence of other symptoms (fever, weight loss, diarrhoea, hepatomegaly, and respiratory symptoms), full blood count, CD4 count, erythrocyte sedimentation rate (ESR), and liver profile were recorded. Chest radiograph and abdominal ultrasound examination were undertaken in most patients. In the presence of diarrhoea, stool samples were examined for evidence of bacterial or parasitic infection. Upper gastrointestinal endoscopy, sigmoidoscopy, and liver biopsy were performed when indicated. Postmortem examinations were obtained, when possible, in patients who died without an established reason for their abdominal pain.

Diagnostic criteria

Disseminated *M. tuberculosis* infection was defined by positive mycobacterial blood or bone marrow cultures, positive mycobacterial cultures from two or more other sites, or postmortem evidence. Atypical mycobacterial infection was diagnosed by mycobacterial blood culture and niacin slope. A diagnosis of gastrointestinal CMV disease was made by demonstration of typical histologic findings in endoscopic biopsy specimens (four of six) or compatible gastrointestinal symptoms in the presence of CMV retinitis with no other identifiable cause. Cryptosporidiosis was diagnosed by visualization of oocysts in stool, duodenal aspirate, or biopsy. The presence of HIV cholangitis was confirmed by endoscopic retrograde cholangiopancreatography (ERCP). Oesophageal ulcers were considered to be HIV-related only when endoscopic biopsy specimens were negative for CMV, herpes simplex, and other known pathogens.

Table I. Demographic features of the study population ($n = 44$)

	Male	Female	Homosexual
Black, *n*	10	15	0
Mixed race, *n*	7	3	4
White, *n*	9	0	9

Table II. Conditions associated with abdominal pain in advanced human immunodeficiency virus (HIV) infection (CD4 < 200) in Cape Town, South Africa ($n = 44$), in accordance with racial origin*

	Total	Black	Mixed race	White
Disseminated tuberculosis	14 (32%)	9	5	0
Cytomegalovirus infection	6 (14%)	1	1	4
Cryptosporidiosis	6 (14%)	4	0	2
HIV cholangitis	3 (7%)	1	0	2
Bacterial enteritis	3 (7%)	3	0	0
Helminths	2 (5%)	2	0	0
Atypical mycobacteria	2 (5%)	0	0	2
HIV-related ulcers	2 (5%)	1	0	1
Acalculous cholecystitis	1 (2%)	1	0	0
Microsporidiosis	1 (2%)	1	0	0
Isosporiasis	1 (2%)	1	0	0

* More than 1 condition in 10 subjects.

Data analysis

Data were entered onto a computer database (Epi Info 6.02). The association between the predominant site of abdominal pain, weight loss, fever, diarrhoea, respiratory symptoms, hepatomegaly, and abdominal ultrasound findings and the presence or absence of disseminated *M. tuberculosis* infection was examined using two-tailed chi-squared analysis. Student's *t* test was used to determine whether there was a relationship between haemoglobin or ESR levels and disseminated *M. tuberculosis* infection. The sensitivity and specificity of abdominal ultrasound findings for predicting disseminated *M. tuberculosis* were calculated. Numbers were not large enough to examine these relationships for other conditions.

Cumulative survival was calculated for all subjects with HIV-related abdominal pain and for the subgroups with and without disseminated *M. tuberculosis*, using the Kaplan–Meier method. This was compared with the survival of all AIDS patients (from the time of AIDS-defining illness) attending the Somerset Hospital HIV clinic during the same time period as the study.

RESULTS

The demographics of the 44 patients studied are shown in Table I. The mean age of the patients was 32.9 (range, 18.4 to 53.3 years). The mean CD4 count was 55 (interquartile range, 11–86); there was no significant difference between the CD4 counts of those with disseminated tuberculosis and those without: 69 (34–93) and 48 (8–58), respectively. Forty subjects (91%) fulfilled the WHO clinical criteria for AIDS (8). No patient underwent surgery during the study period.

A probable cause of the abdominal pain was established in 37 of the 44 patients (84%), with more than 1 cause in 10 (23%) (Table II). Disseminated *M. tuberculosis* infection was the commonest condition, occurring significantly more often in non-white persons ($P < 0.05$). A further three patients were suspected of having disseminated *M. tuberculosis*, but the

criteria for diagnosis were not fulfilled; two others developed abdominal pain while taking antituberculous therapy (these five subjects were not included in the disseminated tuberculosis group for statistical analysis). In contrast, atypical mycobacterial infection was rare and documented in only two cases, both white homosexual men.

Gastrointestinal CMV disease (oesophagitis, gastritis, and duodenitis) was thought to be the cause of abdominal pain in six subjects, four of whom were white. Of the six patients presenting with abdominal pain and cryptosporidiosis, five had co-existing conditions that might have contributed to the pain; three had ERCP evidence of HIV cholangitis, and 2 had bacterial gastrointestinal infections. One case each of microsporidiosis and isosporiasis were found in association with generalized abdominal pain.

A pathologic condition of the oesophagus was responsible for some cases of abdominal pain. Oesophageal ulceration was documented at endoscopy in five subjects and on barium swallow in a further two. There was histologic evidence of CMV disease in two cases and oesophageal tuberculosis in one. Oesophageal biopsies in the other two subjects showed non-specific inflammation, and the symptoms resolved with steroids (HIV-related ulcers).

Thirteen subjects had generalized abdominal pain, 10 pain in more than one area, 6 epigastric pain, 10 right upper quadrant pain, and 5 pain at other sites. There was a trend for generalized abdominal pain to be present more often in those with disseminated *M. tuberculosis* ($P = 0.07$), but otherwise the site of pain was not predictive of the underlying disorder. Thirty-nine patients (89%) had lost weight, 34 (77%) had fever, 31 (70%) diarrhoea, 28 (64%) hepatomegaly, and 20 (45%) respiratory symptoms. The presence of fever, respiratory symptoms, and hepatomegaly were significantly associated with disseminated *M. tuberculosis* infection (all, $P < 0.05$). Haemoglobin and ESR levels did not differentiate between patients with and without disseminated *M. tuberculosis* infection. Liver profiles were often abnormal but not predictive of the underlying disorder.

Chest radiographs taken within 1 month of entry to the study were available for evaluation in 34 subjects (10 with confirmed or suspected disseminated *M. tuberculosis* and 24 with other conditions). Only one patient with disseminated *M. tuberculosis* had a normal radiograph; pleural effusions, atypical infiltrates, reticulonodular pattern, or adenopathy was present in the others. Fifteen (63%) of the patients without disseminated tuberculosis had entirely normal radiographs, two had old fibrocystic changes only, and five (33%) had atypical infiltrates. Pleural effusions, reticulonodular pattern, and adenopathy were seen only in the presence of disseminated *M. tuberculosis* infection.

Ultrasound examination of the abdomen was performed in 35 patients (80%) and was normal in 7 (20%). The main features encountered were liver abnormalities (increased echogenicity or hepatomegaly), abscesses (splenic in four of five cases), adenopathy, ascites, and abnormalities of the gallbladder and biliary system (thickened gallbladder wall, stones, and dilation of the ducts). Splenic size was not recorded prospectively. The presence of intra-abdominal adenopathy, abscesses, or ascites was significantly associated with ($P < 0.05$) and highly specific for disseminated *M. tuberculosis* infection (Table III). When the three patients with suspected disseminated *M. tuberculosis* were included with the proven cases, the specificities of adenopathy and ascites increased to 95%, and abscesses to 100%. Adenopathy and ascites on ultrasound were otherwise seen only in atypical mycobacterial infection.

Table III. Abdominal ultrasound findings in patients with abdominal pain in advanced human immunodeficiency virus infection (CD4 < 200) in the presence (TB) or absence (non-TB) of disseminated *Mycobacterium tuberculosis* infection

Finding	TB ($n = 12$)	Non-TB ($n = 23$)	Sensitivity for TB	Specificity for TB
Hepatic abnormalities	9	10	75%	57%
Adenopathy	8	2	67%	91%
Abscesses	4	1	33%	96%
Ascites	5	2	42%	91%
Bile duct dilatation	1	7	NA	NA
Gallstones	1	1	NA	NA
Cholecystitis	0	1	NA	NA

Stool samples from 18 subjects were sent for microscopy and culture, with identification of possible pathogens in 7 (*Trichuris trichiura* in 3, *Campylobacter jejuni* in 1, *Shigella sonnei* in 1, *Cryptosporidium parvum* in 2, *Isospora belli* in 1, and *M. tuberculosis* in 1). Upper intestinal endoscopy with biopsies in 17 patients yielded a diagnosis in 12 (71%): conditions found were CMV-related disease, cryptosporidiosis, microsporidiosis, oesophageal candidiasis, disseminated *M. tuberculosis*, HIV-related oesophageal ulcers, and gastritis. ERCP was performed in five subjects with evidence of dilated bile ducts on ultrasound. HIV cholangitis was confirmed in three, whereas one procedure failed, and the other showed a normal biliary tree. Hepatic biopsy (two cases) and sigmoidoscopy (five cases) did not increase diagnostic yield. Seven postmortem examinations were performed; five showed disseminated mycobacterial infection (four *M. tuberculosis* and one atypical), one cryptosporidiosis and cryptococcosis involving the small bowel, and one was non-diagnostic.

Twenty-nine subjects died during the follow-up period, with a median time to death of 101 days (interquartile range, 56–210 days). Six patients died within 1 month of entering the study; five had disseminated mycobacterial infection, and one disseminated CMV disease. The median follow-up time of the survivors was 168 days (81–262 days). Cumulative survival of the abdominal pain cohort was 51% (standard error of the mean ($s_{\bar{x}}$) ± 8%) at 6 months, compared with 73% ($s_{\bar{x}}$ ± 3%) for Cape Town AIDS patients ($P < 0.001$) (Fig. 1). There was no significant difference between the cumulative survival at 6

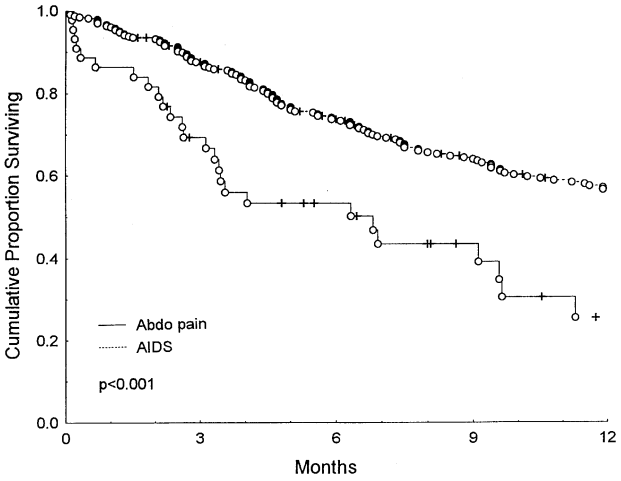

Fig. 1. Cumulative survival rates of subjects with abdominal pain in advanced human immunodeficiency virus infection ($n = 44$) and Cape Town acquired immunodeficiency syndrome (AIDS) patients ($n = 332$). Data were complete (○) in 29 patients with abdominal pain and 194 with AIDS and censored (+) in 15 and 138, respectively.

months of those with disseminated *M. tuberculosis* (58%) and those with other conditions (50%).

DISCUSSION

This study has shown that the aetiology of HIV-related abdominal pain in South Africa differs considerably from that reported in industrialized countries. To our knowledge, this is the first prospective study of an unselected group of patients with abdominal pain in advanced HIV infection, and it is certainly the first such study to be reported from Africa. Previous data have largely been derived from small surgical series and retrospective data analysis.

The study was designed to capture all known patients with advanced HIV infection presenting to Somerset Hospital, Cape Town, with abdominal pain. Most presented to the HIV physicians, but patients with symptomatic HIV disease presenting to the surgeons are usually managed with the physicians and should, therefore, have been identified. Whereas the increasingly high prevalence of HIV infection in the local African community (around 10%) makes it likely that some unidentified seropositive subjects will have been missed, we believe that the study findings are representative of the Cape Town HIV population and are probably generalizable to HIV-infected persons in the rest of South Africa and other non-industrialized countries.

In contrast to previous series, no patient underwent surgery during the study period. This can be partly attributed to the fact that suspected tuberculous peritonitis was managed by the physicians and not referred to the surgeons. However, there was no evidence of bowel perforation and obstruction among the study patients even at postmortem, suggesting that these conditions occur less commonly in the Cape Town HIV population. It is unlikely that sick patients were underrepresented in the series, as Somerset Hospital is a regional HIV referral centre, and mortality during the study period was high.

In non-industrialized countries it is important to have simple cost-effective management strategies for common problems like HIV-related abdominal pain. Epidemiologic data are very important in this setting, both to document the local prevalence of conditions and to identify clinical markers for the different disease processes.

Abdominal tuberculosis was the commonest cause of abdominal pain in non-white persons with advanced HIV infection in Cape Town, reflecting the high prevalence of tuberculosis in these communities. Abdominal involvement has been found to be universal in HIV-infected persons with disseminated *M. tuberculosis* infection (9). Unfortunately, postmortem data in this study were limited, owing to logistic and religious difficulties in obtaining permission to perform examinations on non-white subjects, but tuberculous peritonitis was demonstrated in all four cases of disseminated *M. tuberculosis* for which postmortem data were available. It is important to recognize that disseminated tuberculosis may present with an acute abdomen, since optimal management is immediate antituberculous therapy, to which there is usually an excellent response, and surgery should be reserved for complications (10).

It was not possible to show a clear relationship between the predominant site of pain and the underlying aetiology for two main reasons. First, about a quarter of the patients studied had multiple pathologic conditions, making it difficult to determine the relative contribution of each to the symptoms, and, secondly, the numbers with any particular condition other than disseminated tuberculosis were small. Interestingly, most of the subjects with disseminated *M. tuberculosis* did not have co-existing conditions and usually presented with generalized abdominal pain, possibly related to tuberculous peritonitis.

The presence of fever, hepatomegaly, respiratory symptoms, or an abnormal chest radiograph in an HIV patient with abdominal pain was predictive of a diagnosis of disseminated *M. tuberculosis*. Computerized tomography of the abdomen has been shown to be of diagnostic value in abdominal tuberculosis, with adenopathy being the commonest finding (11, 12), but abdominal ultrasound examination, which is widely available and non-invasive, can provide the same information in most cases. We have shown that adenopathy, ascites, or abscesses are strongly associated with disseminated tuberculosis and would suggest that these findings together with the clinical presentation are sufficient to justify empiric antituberculous therapy pending appropriate mycobacterial culture results.

Abdominal pain related to atypical mycobacterial infection was diagnosed in two white homosexual men only, consistent with the previously reported low prevalence of atypical mycobacterial infection in African AIDS patients (7, 13). An

inverse relationship between the prevalence of *M. tuberculosis* and atypical mycobacterial infection has been noted (14), and it is possible that previous exposure to *M. tuberculosis* or bacillus Calmette–Guérin vaccination has a protective effect. However, HIV-infected Africans who have emigrated to the West have been found to develop atypical mycobacterial infections with the same frequency as locals, suggesting that environmental factors are also involved (15).

CMV disease of the gastrointestinal tract was relatively common in the Cape Town HIV population. Earlier reports suggested that CMV disease was uncommon in African AIDS patients (16), but it is being increasingly recognized in non-white persons, which is in keeping with preliminary findings of a high seroprevalence for CMV in Africans in Cape Town.

Cryptosporidium parvum has been isolated from 25–50% of patients with prolonged diarrhoea and wasting in Central Africa ('slim disease') (17, 18). Chronic diarrhoea is less of a problem in South African AIDS patients (4), but when it occurs, is often related to cryptosporidiosis. Cryptosporidiosis has previously been reported to cause diffuse abdominal pain (2, 5), but more localized pain would suggest the presence of additional pathologic conditions, such as secondary cholangitis or other infections, as was found in five of the six patients in this series. Similarly, *Isospora belli* and microsporidial infection might be responsible for diffuse abdominal pain, as was found in the two cases reported in this study.

Although the relatively small size of this series might explain the failure to show gastrointestinal lymphoma as a cause of abdominal pain in South African HIV patients, there have been no cases of HIV-related gastrointestinal lymphoma recorded in Cape Town since the start of HIV data collection in 1984; it would therefore appear to be as uncommon in South Africa as it has been reported to be in India (19).

None of the patients studied in Cape Town were receiving dideoxyinosine (ddI), but it is still surprising that pancreatitis was not identified as a cause of abdominal pain, as it has been shown to occur more frequently in HIV-infected persons, even in the absence of ddI (20).

The occurrence of abdominal pain in AIDS patients has previously been shown to carry a poor prognosis (2). The 6-month survival of our patient cohort was significantly decreased as compared with Cape Town AIDS patients in general. There are two possible explanations for this increased mortality. First, the abdominal pain is due to a condition such as intestinal perforation, which carries a high mortality in itself, and secondly, the abdominal pain may be associated with advanced immunocompromise. The latter explanation seems more likely, as no acute surgical emergencies were identified in our series, and the patients who died within a month of entering the study all had evidence of disseminated opportunistic infection. *M. tuberculosis* infection in HIV-infected patients is generally expected to respond well to antituberculous therapy, but inability to localize the disease may be associated with a poor prognosis.

In conclusion, the commonest cause of abdominal pain in advanced HIV infection in Cape Town was tuberculosis, reflecting its high local prevalence. Cryptosporidial and CMV infection were relatively common, but atypical mycobacterial infection was rare and only seen in white homosexuals. Gastrointestinal lymphoma and pancreatitis, which are leading causes of AIDS-related abdominal pain in the West, were not found in Cape Town.

Abdominal ultrasound examination and upper gastrointestinal endoscopy were found to be valuable diagnostic tools. The symptom complex of abdominal pain in association with fever, hepatomegaly, respiratory symptoms, or an abnormal chest radiograph was found to be highly suggestive of tuberculosis, and the presence of adenopathy, ascites, or intra-abdominal abscesses on ultrasound should be considered sufficient justification for initiating antituberculous therapy pending appropriate mycobacterial culture results. The occurrence of abdominal pain in advanced HIV infection usually implies underlying disseminated opportunistic infection and carries a poor prognosis.

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AIDS IN AFRICA — SURVIVAL ACCORDING TO AIDS-DEFINING ILLNESS

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Objective. Evaluation of prognostic significance of the type of AIDS-defining illness (ADI) and performance status in a cohort of AIDS patients.

Design, setting, subjects, outcome measures. A retrospective analysis of 280 patients with AIDS, as defined by the proposed World Health Organisation (WHO) clinical staging system, who attended two Cape Town-based HIV clinics between 1984 and 1997. Patients were stratified according to the type of initial ADI. Survival associated with each opportunistic event was determined by Kaplan-Meier analysis. Cox proportional hazard analysis was used to determine relative risk for death associated with three strata of ADI.

Results. Median survival associated with various initial ADIs varied from less than 3 months (encephalopathy and wasting), to over 2 years (extrapulmonary tuberculosis and herpes simplex virus infection). This effect of ADI on outcome was most striking in patients with relatively preserved CD4 counts (CD4 > 50/μl). A performance status score 4 predicted 50% mortality at 1 month, irrespective of co-morbidity.

Conclusion. The type of ADI is an important determinant of survival, particularly in patients with preserved CD4 counts. The stratification of patients by type of ADI and performance status may be useful in the management of patients with advanced HIV infection in resource-limited environments.

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The disease burden caused by HIV infection has been overwhelming health care facilities in many African countries.[1] It has been suggested that African patients with HIV infection have an increased rate of progression from asymptomatic HIV infection to AIDS compared with patients in the developed world, and this difference has been related to limited access to

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medical care.[1] However, a recent longitudinal cohort study from Uganda[2] showed the rate of progression to AIDS in HIV-positive patients to be comparable to figures previously reported from the Western world. Survival of South African AIDS patients, provided with adequate medical care, has been shown to be similar to that of European and American patients with AIDS.[3]

Laboratory markers that reflect the degree of immune compromise in HIV infection, such as CD4+ T-lymphocyte (CD4) count[4] and quantitative HIV viral load,[5] are frequently unavailable in Africa. In this setting, prognostic information may be obtained from clinical staging as proposed by the World Health Organisation (WHO) clinical staging system and the total lymphocyte count.[2,6,7]

AIDS includes a variety of opportunistic diseases that may occur across a spectrum of immune dysfunction.[8] The prognosis for patients with AIDS varies accordingly and is influenced significantly by the type of AIDS-defining illness (ADI).[3,9,10] Prognostic stratification based on ADI and performance status can be performed in the outpatient clinic and does not require sophisticated laboratory facilities. It is therefore widely applicable in resource-poor settings, and potentially useful to guide resource allocation and patient management. This study evaluated outcome of the commonest AIDS-defining events and poor performance status in a cohort of South African HIV-positive patients.

METHODS

All patients with an AIDS diagnosis (as defined by the proposed WHO clinical staging system,[11] which is similar to the Centers for Diseases Control 1987 definition of AIDS[12]) attending the HIV outpatient clinics of the University of Cape Town medical school (at Somerset and Groote Schuur hospitals) were selected from computer-based medical records. From 1984 to 1997, patients with HIV infection were regularly followed up at these clinics at 6-monthly intervals during the asymptomatic phase of their illness, more frequently as opportunistic infections occurred. At each visit, patients were staged according to the WHO clinical staging system, retrospectively until 1992, prospectively thereafter,[3] and CD4 counts were regularly performed. Tertiary care diagnostic facilities such as bronchoscopy, liver and bone marrow biopsies, microbiology and computed tomography (CT) scanning, were available to confirm the presence of AIDS-related illnesses. These clinics mainly served the underprivileged communities, where heterosexually acquired HIV infection had been increasingly prevalent. However, before 1991 the majority of clinic attendees had been white male homosexuals.[3]

Patients with AIDS or CD4 counts below 200/μl routinely received co-trimoxazole prophylaxis since 1996, and standard treatment for tuberculosis (TB), herpes simplex infection (HSV), oesophageal candidiasis, *Pneumocystis carinii* pneumonia (PCP) and cryptococcal meningitis was available. Antiretroviral therapy was not routinely available for patients with AIDS, and 48 patients who had received such therapy were excluded from analysis.

Date of death was obtained from hospital records or from deaths reported to the clinics by relatives or friends. In addition, regional death records were searched if patients failed to attend for more than 6 months. Status of patients discharged from the clinic for terminal care was obtained from the Red Cross Home-Based Care Society.

Survival was analysed from the first presentation at either clinic according to type of ADI, for diseases that occurred in at least 10 patients. If two or more ADIs occurred simultaneously, patients were placed in the group according to the event with the worst outcome. Survival of patients with AIDS was also analysed according to CD4 count (< 50, 51 - 200, > 200/μl), and for patients with performance status 4 (in bed > 50% of normal day time during the last month). CD4 counts performed within 3 months (before or following) onset of AIDS-related illnesses were used for analysis.

Survival was calculated in months from the index visit (first visit at which the condition occurred) to the date of death or last visit (censored), using Epi-info (version 6). Kaplan-Meier survival curves were created using the software package Statistica (version 6), and evaluated for statistical difference by log-rank test. Relative hazards of death and 95% confidence intervals (CIs) were calculated using the univariate Cox proportional hazard model and Fisher's exact test.

RESULTS

From May 1984 to April 1997, 1 735 patients with HIV infection were seen during 11 493 visits. By April 1997, 280 patients had developed AIDS, and 160 of this number had died. Of the 120 patients alive at the end of the study, 45 had been lost to follow-up after a median clinic attendance of 8.7 months (range 1 - 63 months). Median follow-up for patients with AIDS who were still alive at the end of the study period was 10.2 months (range 1 - 65 months), and 9.7 months (0 - 114) for those who had died. The median number of clinic visits for patients with AIDS was 5 (range 1 - 47), the mean age of patients was 33 years (range 17 - 75 years), and both homosexual (N = 109) and heterosexual (N = 171) transmission patterns, male (N = 199) and female (N = 81) gender, and the three major population groups (76 whites, 75 coloureds and 129 blacks) were represented. Intravenous drug abuse and haemophilia did not occur as risk factors for HIV infection in our patients. Both patients who were diagnosed HIV-positive on presentation at either clinic with an ADI (N = 143) and patients who developed AIDS during follow-up from WHO clinical stages 1 - 3 (N = 137) were represented in this cohort. The total number of ADIs diagnosed in our cohort was 430 (average per patient 1.54); the

eight diseases mentioned in Table I occurred 317 times (261 as initial ADI).

The overall median survival time from the onset of AIDS was 11.5 months. Initial ADIs were stratified into early, intermediate and late events, according to median survival rates (Table I). There was no statistical difference for the survival curves of individual diseases within each stratum ($P = 0.90$, 0.43, and 0.98 respectively). Kaplan-Meier survival for each of the three strata of opportunistic diseases is depicted in Fig. 1. Performance status score 4 predicted 50% 1-month mortality, irrespective of co-morbidity.

Survival of AIDS patients was related to both CD4 count and ADI. The relative risk of death for patients with early, intermediate and late diseases within defined strata of CD4 counts is shown in Table II. The influence of ADI on mortality was most striking in patients with relatively preserved CD4 counts.

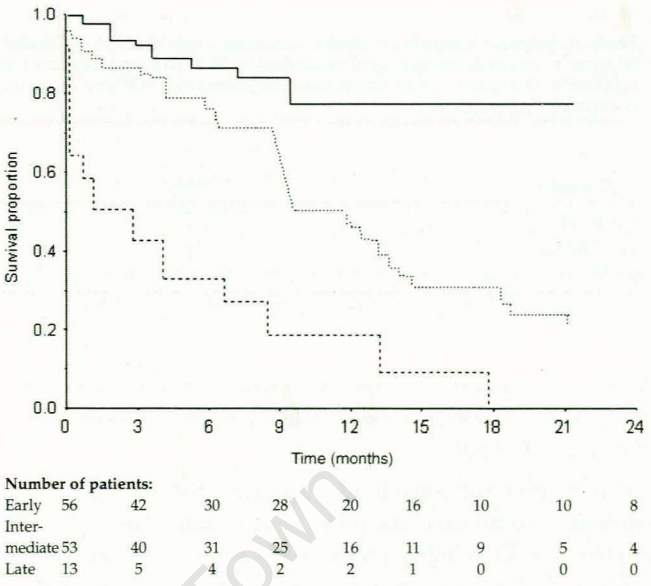

Fig. 1. Survival for the eight commonest AIDS-defining illnesses could be stratified into one of three patterns: early diseases include extrapulmonary TB and HSV, intermediate diseases KS, oesophageal candidiasis, PCP and cryptococcal meningitis, late diseases encephalopathy and wasting. Survival was significantly different for early (solid line), intermediate (dotted line) and late events (intermittent line) (P < 0.00001).

Discussion

This study, undertaken in a resource-limited environment, showed that the type of ADI was a major predictor of outcome, and that the opportunistic disease could be used as a prognostic adjunct to the CD4 count. Stratification of patients according to ADI and performance status is easily performed, and therefore widely applicable in resource-poor settings. The prognostic information provided by these clinical parameters can be used for counselling and management of HIV-infected patients.

Extrapulmonary TB and HSV infection (lasting more than 1 month) as initial AIDS diagnoses were associated with the most favourable outcome and survival was comparable to reported figures from the developed world.[9,10] Extrapulmonary TB was the initial AIDS diagnosis in one-third of our patients, and was associated with a relatively preserved CD4 count. Our diagnostic facilities allowed for early detection and treatment of extrapulmonary TB, which may explain the more favourable overall prognosis for patients with AIDS in Cape Town compared with Uganda.[2,3]

Survival in our patients following cryptococcal meningitis was similar (7 v. 9 months) to survival of patients with cryptococcosis in a large European study,[10] and predicted outcome for Kaposi's sarcoma (KS) was similar to that in an American cohort (12 v. 12.3 months).[13] The comparability of

Table I. Median CD4 counts and survival (Kaplan-Meier estimates) for the commonest initial AIDS-defining illnesses

AIDS-defining illness	Median CD4 count (range)	Median survival (mo. from diagnosis)	12-month survival (%)	Patients (*N*)
Early	111	> 24	68	116
Extrapulmonary TB	111 (5 - 990)	> 24	71	97
Herpes simplex virus	114 (8 - 294)	> 24	66	19
Intermediate	48	9	44	105
Kaposi's sarcoma	118 (10 - 581)	12	58	24
Oesophageal candidiasis	76 (1 - 403)	9	52	32
Pneumocystis carinii pneumonia	39 (8 - 155)	7	39	38
Cryptococcal meningitis	32 (9 - 200)	7	23	11
Late	64	2	23	40
Encephalopathy	121 (9 - 393)	3	21	20
Wasting	45 (1 - 755)	1	24	20

Initial AIDS-defining illnesses were stratified into early, intermediate and late events according to median survival rates (> 12, 6 - 12, < 6 months). *N* reflects the number of patients in each stratum, and median survival is expressed in months from diagnosis.

Table II. Relative hazards for death associated with class of AIDS-defining illness and defined CD4 count strata. Values for intermediate (Kaposi's sarcoma, oesophageal candidiasis, PCP and cryptococcal meningitis) and late diseases (encephalopathy and wasting) are expressed relative to the risk of early diseases (extrapulmonary TB and herpes simplex) in the same CD4 count stratum. Figures in brackets represent 95% confidence intervals

	Class of AIDS-defining illness		
CD4 count	Early	Intermediate	Late
> 200/µl	1	10.50 (1.60 - 88.90)	21.00 (1.83 - 240.50)
51 - 200/µl	1	3.18 (1.98 - 4.70)	4.33 (1.88 - 30.90)
0 - 50/µl	1	1.39 (0.50 - 3.56)	2.90 (1.60 - 5.00)

African and Western AIDS survival figures supports the notion that access to care is an important determinant of the survival of African AIDS patients.[1]

Reports from sub-Saharan Africa suggest that wasting syndrome is common,[1,2] and post-mortem studies have revealed that TB is highly prevalent in cachectic African AIDS patients.[14] HIV-positive patients who presented to our clinics with a wasting illness were thoroughly investigated for TB by means of sputum smears and culture, histology of lymph node, liver or bone marrow, and blood culture. The resulting high frequency with which TB was diagnosed, and the relatively low prevalence of diarrhoeal illnesses in South African HIV-positive patients,[15] may account for the small number of patients with unexplained HIV-wasting syndrome in this study.

The type of ADI,[9,10] prior HIV or AIDS-related morbidity,[16] total lymphocyte count[5,6] and performance status[17] all provide useful prognostic information in patients with advanced HIV infection. This study has grouped opportunistic illnesses into three categories, as cases were recruited from a single site and hence numbers for individual diseases were small. Although the survival difference for the three groups was marked, and the outcome for each group is consistent with our clinical observations, we cannot exclude the possibility that these differences occurred by chance because of *post hoc* classification. Our stratification would therefore need to be validated prospectively in an African setting.

Prognostic stratification of HIV-infected patients is particularly relevant in resource-poor countries in order to avoid irrational spending of scarce health care resources. Expensive investigations or therapy could then be limited to patients with favourable prognostic criteria. Survival figures of patients with performance status 4 or diseases such as HIV wasting syndrome or encephalopathy, on the other hand, supports the institution of home-based terminal care for these patients.

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THE SPECTRUM AND PROGNOSIS OF AIDS-DEFINING ILLNESSES IN CAPE TOWN

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Objectives. To describe the incidence, spectrum and prognosis of AIDS-defining illnesses (ADI) in patients without access to antiretroviral therapy (ART).

Design. Prospective cohort study.

Subjects. 1 215 HIV-infected patients attending adult HIV clinics affiliated to the University of Cape Town in the New Somerset and Groote Schuur Hospitals from 1992 to 2000.

Main outcome measures. Incidence rate (IR) of ADIs and survival after the development of ADI.

Results. During follow-up, 430 ADIs occurred (IR = 21.3 cases per 100 patient-years (PYs)). IR varied according to CD4 count, with 38.8, 17.0 and 8.52 cases/100 PYs in patients with CD4 counts < 200 cells/µl, 200 - 350 cells/µl and > 350 cells/µl, respectively. Tuberculosis (TB) was the commonest ADI, followed by candidiasis of the oesophagus/trachea/bronchi. IRs for chronic herpes simplex ulcers, HIV wasting, *Pneumocystis carinii* pneumonia and Kaposi's sarcoma were > 1.00 cases/100 PYs. TB was diagnosed in all CD4 strata, and was the only illness to occur commonly above 200 cells/µl. The median CD4 counts within 6 months of diagnosis of ADI ranged from 138 cells/µl for TB to 17 cells/µl for cryptococcosis. Overall, median time to death from date of diagnosis was 18 months, and ranged from 24.1 months for patients diagnosed with TB to 6 months for those diagnosed with cytomegalovirus.

Conclusions. HIV-infected patients with no access to ART in Cape Town are at high risk of AIDS-defining illnesses. This study provides useful data for designing therapeutic interventions for preventing these infections.

HIV infection is characterised by progressive immune suppression, which eventually results in the development of opportunistic diseases.[1-3] The spectrum of these infections varies across the world, and is partially determined by the organisms that are prevalent in the community.

The spectrum of severe HIV-related diseases in sub-Saharan Africa is characterised by an abundance of virulent pathogens such as *Mycobacterium tuberculosis, Streptococcus pneumoniae* and *Salmonella* spp.[4,5] *Pneumocystis carinii* pneumonia (PCP) is uncommon, and avirulent organisms such as cytomegalovirus (CMV) and *M. avium* complex are seldom found.[4,5] Tuberculosis (TB), bacterial infections and malaria are the leading causes of HIV-related morbidity across sub-Saharan Africa. In studies reporting CD4 count, the range of cell counts at the time of diagnosis of severe HIV-related diseases is wide.[4,5]

While the spectrum of severe HIV-related diseases in industrialised countries is well described, few published studies exist describing the incidence and prognosis of HIV-related diseases in Africa, and the level of immune suppression at which these diseases occur. Most existing studies are of cross-sectional design, and is difficult to compare them owing to differences in referral patterns, study population, criteria for HIV testing, diagnostic methods, and the disease reported.[6] Sound incidence data on severe HIV-related events are important for designing preventive or curative therapeutic intervention.

METHODS

The objectives of this analysis were to estimate the incidence of AIDS-defining illness (stratified by CD4 count and WHO clinical stage) and survival after the development of AIDS-defining illness, and to describe the distribution of the CD4 count within 6 months of the onset of AIDS-defining illnesses in HIV-infected patients with no access to antiretroviral therapy (ART).

The analysis was based on the Cape Town AIDS Cohort (CTAC), which comprises patients from the wide socio-demographic

spectrum of the Western Cape. CTAC is based on patients attending public sector HIV health care services at New Somerset Hospital (NSH) and Groote Schuur Hospital (GSH), which are affiliated to the University of Cape Town. For the purpose of this analysis, patients presenting with an AIDS-defining illness at their initial clinic visit or receiving any form of ART were excluded.

Data on the CTAC patients were collected prospectively from 1992. Routine demographic, clinical and laboratory data were collected using a standard computerised format. Patients presented to the clinic approximately 3 - 6-monthly or more frequently if clinically indicated. At each visit, patients were examined for manifestations of HIV/AIDS-related illness and staged using the World Health Organization (WHO) criteria.[7] HIV diagnosis was confirmed by enzyme-linked immunosorbent assay (ELISA) and confirmed with Western blot (or subsequently a second ELISA) on two different blood specimens. The CD4 count was measured approximately bi-annually, using flow cytometry (Beckman Coulter®, Miami, Fla, USA). Vital status data were acquired from the inpatient records, via notification by family or the patient's general practitioner, or by reviewing local death registries. The analysis was carried using Epi Info (version 6.0; CDC, Atlanta, Ga, USA) and STATISTICA (release 6.6, Tulsa, Oklahoma, USA).

AIDS-FREE SURVIVAL AND RISK OF DEATH FROM ONSET OF AIDS

The Kaplan-Meier method was used to plot AIDS-free survival curves by WHO stage and CD4 count from the initial clinic visit date to the date of onset of an AIDS-defining illness. The same method was used to measure risk of death from the onset of AIDS. The analysis was further stratified by specific illness. CD4 count was measured ± 6 months from the initial clinic visit, if not available at the date of the initial clinic visit. CD4 count was categorised as < 200, 200 - 350 and > 350 cells/µl.

INCIDENCE OF AIDS-DEFINING ILLNESSES

The incidence rate (IR) for each diagnosis was calculated by dividing the number of new diagnoses of the AIDS-defining illness that occurred after entry into the cohort by the total number of the person-years (PYs) of the cohort. The analysis was further stratified by baseline CD4 count. The time spent in each CD4 count stratum was summed, yielding PYs by CD4 count stratum, and the incidence rate of each AIDS-defining illness was calculated as the number of new cases occurring while in a given CD4 count stratum, divided by the total number of PYs of observation in that CD4 count stratum. For each AIDS-defining illness the distribution of CD4 counts (within ± 6 months of the diagnosis of the condition) was examined. All-cause mortality was estimated for each AIDS-defining illness using the Kaplan-Meier method. Follow-up was continued until death, date last seen in the clinic or end-point of the study (31 December 2000). In this analysis, all sites of TB diagnosis (pulmonary, extrapulmonary or disseminated) were included in one group.

RESULTS

STUDY SAMPLE

The study included 1 215 patients who did not use ART during follow-up and had no AIDS-defining illness before the initial clinic visit. The baseline characteristics of these patients are described in Table I. The cohort consists mostly of young indigent patients with a wide clinical and immune suppression spectrum. Approximately 50% of these patients received prophylactic cotrimoxazole at some point of their follow-up.

TABLE I. BASELINE CHARACTERISTICS OF THE COHORT

Age (mean ± SD) (yrs)	31.3 (± 8.89)
CD4 count	
< 200 cells/µl	415 (34.2%)
200 - 350 cells/µl	319 (26.3%)
> 350 cells/µl	446 (36.7%)
Not done	35 (2.8%)
Median (interquartile range)	276.5 (143.5 - 437.0)
WHO stage	
Stage I	547 (45%)
Stage II	210 (17.3%)
Stage III	458 (37.7%)
Use of cotrimoxazole	644 (53.0%)
Gender	
Female	634 (52.2%)
Male	581 (47.8%)
Socioeconomic status	
Low	872 (71.8%)
High	343 (28.2%)

SD = standard deviation.

AIDS-FREE SURVIVAL

Median time to occurrence of an AIDS-defining illness varied according to baseline CD4 count (ranging from 63 months for patients with CD4 count > 350 cells/µl to 18 months in those with CD4 count < 200 cells/µl) and WHO stage (ranging from 56 months for patients with WHO stage I to 18 months in those with WHO stage III) (Fig. 1).

INCIDENCE OF AIDS-DEFINING ILLNESSES

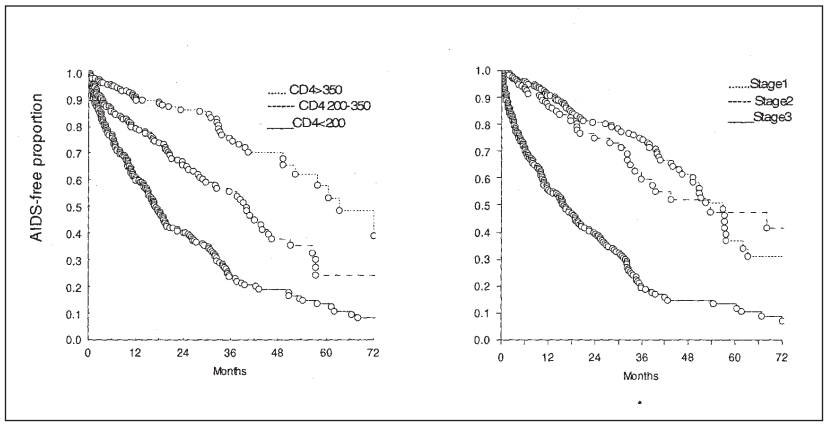
During a median 1.66 years of follow-up, 430 AIDS-defining illnesses occurred (IR = 21.3 cases per 100 PYs, 95% confidence interval (CI) 19.6 - 23.2) (Table II). Of these illnesses, 255 occurred in patients with CD4 count < 200 cells/µl (IR = 38.8 cases per 100 PYs, 95% CI 35.0 - 42.6), 108 illnesses in patients with CD4 count 200 - 350 cells/µl (IR = 17.0 cases per 100 PYs, 95% CI 14.2 - 20.2), and 59 in patients with CD4 count > 350 cells/µl (IR = 8.52 cases per 100 PYs, 95% CI 6.6 - 10.9). Eight illnesses occurred in 30.1 PYs of follow-up in patients with unknown CD4 count (IR = 26.7, 95% CI 12.3 - 45.9 cases per 100 PYs). Incidence ranged from 10.6 cases per 100 PYs for TB to 0.05 cases per 100 PYs for endemic mycosis.

CD4 COUNT AT DIAGNOSIS OF AIDS-DEFINING ILLNESSES

At (or ± 6 months of) the date of diagnosis, median CD4 counts ranged from 138 cells/µl for TB cases to 17 cells/µl for cryptococcosis (Fig. 2).

SURVIVAL FOLLOWING DIAGNOSIS OF AIDS-DEFINING ILLNESSES

Overall, median time to death from date of diagnosis was 18 months. Median survival ranged from 24.1 months for patients diagnosed with TB to 6 months for those diagnosed with CMV (Table III).



Median AIDS-free survival (months)

CD4 > 350	CD4 200 - 350	CD4 < 200	Stage 1	Stage 2	Stage 3
63	41	18	56	39	18

Fig. 1. Kaplan-Meier probabilities of AIDS-free survival according to baseline CD4 count category and WHO stage.

DISCUSSION

This study reported high rates of AIDS-defining illnesses among HIV-infected patients living in Cape Town with no access to highly active antiretroviral therapy (HAART). The IR of these illnesses was high at low CD4 cell strata, particularly in the < 200 cells/µl stratum. TB was the commonest illness, followed by candidiasis of the oesophagus/trachea/bronchi or lungs. Herpes simplex virus (HSV) infection, wasting, *Pneumocystis carinii* pneunomia (PCP) and Kaposi's sarcoma had an IR > 1.00 cases per 100 PYs. Disseminated mycosis, progressive multifocal leukoencephalopathy and lymphoma were rare. TB was diagnosed in all CD4 strata, and was the only illness to occur commonly above 200 cells/µl. This study is one of only a few that have assessed the incidence of the AIDS-defining illnesses in Africa, stratified both by CD4 count and WHO clinical stage. However, the preponderance of TB and the level of immune suppression at which it occurs, and the overall spectrum of illnesses, is consistent with other reports from the region.[5,8-10] Of note is the fact that most of the illnesses diagnosed in this cohort are preventable or are amenable to treatment. The incidence estimates and time to AIDS according to baseline CD4 count and WHO stage, and survival following the onset of each AIDS-

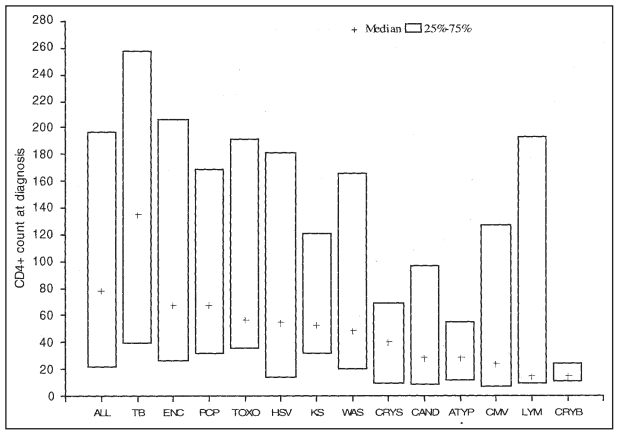
TABLE II. INCIDENCE OF AIDS-DEFINING ILLNESSES IN 1 215 HIV-INFECTED PATIENTS WITH NO ACCESS TO HAART (1992 - 2000) BY CD4 COUNT STRATA

Illness	Overall (2014.8 PYs)		< 200 cells/µl (657.5 PYs)		200 - 350 cells/µl (635.1 PYs)		> 350 cells/µl (692.1 PYs)		N/A
	No.	Incidence	No.	Incidence	No.	Incidence	No.	Incidence	
1 Tuberculosis	214	10.6	111	16.88	62	9.76	39	5.64	2
2 Candidiasis of O/T/B/L	57	2.83	40	6.08	13	2.05	2	0.29	2
3 HSV	27	1.34	19	2.89	7	1.10	1	0.14	0
4 Wasting	27	1.34	15	2.28	6	0.94	6	0.87	0
5 PCP	24	1.19	15	2.28	6	0.94	3	0.43	0
6 Kaposi's sarcoma	23	1.14	17	2.59	2	0.31	3	0.43	1
7 Cryptococcosis	13	0.65	9	1.37	2	0.31	1	0.14	1
8 Encephalopathy	11	0.55	5	0.76	4	0.63	1	0.14	1
9 Cryptosporidiosis	10	0.50	8	1.22	0	0	1	0.14	1
10 Disseminated atypical mycobacteria	8	0.40	5	0.76	3	0.47	0	0	0
11 CMV	6	0.30	4	0.61	1	0.16	1	0.14	0
12 Lymphoma	4	0.20	4	0.61	0	0	0	0	0
13 Toxoplasmosis	3	0.15	3	0.46	0	0	0	0	0
14 PML	2	0.10	0	0	2	0.31	0	0	0
15 Mycosis	1	0.05	1	0.15	0	0	0	0	0
Total	430	21.34	256	38.94	108	17.01	58	8.38	8

Incidence = per 100 PY; Candidiasis of O/T/B/L = candidiasis of the oesophagus, trachea, bronchi or lungs;
PML = progressive multifocal leukoencephalopathy.

TABLE III. KAPLAN-MEIER PROBABILITIES OF SURVIVAL FOLLOWING DIAGNOSIS.

Condition	All	TB	WAS	KS	HSV	PCP	CRYPS	CAND	Enc	Lymph	CRYPO	ATYP	CMV
Median survival	18.1	24.1	22.2	17.8	17	14	13.9	9.5	8.6	7.6	6.5	6.4	6.1



TB = all forms of TB; ENC = HIV encephalopathy; PCP = *Pneumocystis carinii* pneumonia; TOXO = toxoplasmosis; HSV = herpes simplex virus; KS = Kaposi's sarcoma; WAS = HIV wasting syndrome; CRYS = cryptosporidiosis with diarrhoea > 1 month; CAND = candidiasis of the oesophagus, trachea, bronchi or lungs; ATYP = disseminated atypical mycobacteriosis; CMV = cytomegalovirus; LYM = lymphoma; CRYB = extrapulmonary cryptococcosis.

Fig. 2. CD4 count distribution at (or ± 6 months of) the date of diagnosis.

defining illness calculated in this study, are useful data for designing and assessing the outcome of therapeutic interventions for preventing and treating these infections.

Survival after AIDS in Africa may be shorter than in industrialised countries. This may be due to high prevalence of virulent pathogens in the environment and lack of access to health care. In a recent review, Holmes *et al.*[5] observed that in sub-Saharan Africa, TB and bacterial infections are the major cause of morbidity and mortality among hospitalised patients. Bacteraemia, particularly caused by non-typhoid salmonellae and *S. pneumoniae*, and associated with cryptosporidia and *Isospora belli,* are the most frequently isolated pathogens. Non-typhoid salmonellae and *Shigella* species are also commonly isolated when stool cultures are performed. Cerebral toxoplasmosis, and meningitis due to *Cryptococcus neoformans*, tuberculosis and bacterial pathogens, are the most frequent neurological infections. Infections with atypical mycobacteria, *Pneumocystis carinii* and CMV are rare.[5] They also found that, compared with industrialised countries, death occurs at a higher range of CD4 counts, although still in the range consistent with advanced disease.

The findings of this study have important implications for health care management of HIV-infected patients. Very few infections preventable by cotrimoxazole occurred at CD4 counts > 200 cells/µl. This suggests a limited beneficial effect for prophylactic cotrimoxazole in patients with CD4 counts at this level. The high incidence of TB across the different CD4 count strata indicates that preventive therapy with isoniazid should be considered early in the course of HIV disease.

This study had the following limitations. Cause of death was not ascertained, and therefore all-cause mortality was reported. As such, we are unable to exclude non-HIV-related causes of death. Median follow-up of 19.9 months is relatively short and reflects the difficulty of maintaining long follow-up in this setting. It is therefore possible that the actual incidence rate of the various infections reported among this cohort might have been underestimated.

In conclusion, in HIV-infected patients without access to HAART in Cape Town, the overall risk of AIDS-defining illnesses is high and increases with lower CD4 counts and more advanced WHO clinical stage. Early prevention and treatment of these infections will result in large health benefits. Guidelines for therapeutic interventions for prevention of AIDS-defining illness, including ART, in this region should be tailored according to the spectrum and epidemiology of these infections.

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SOUTHERN AFRICAN HIV CLINICIANS SOCIETY

AIDS REVIEW

ADHERENCE TO ANTIRETROVIRAL THERAPY — ACHIEVABLE IN THE SOUTH AFRICAN CONTEXT?

'Patients will fail to take antiretroviral drugs consistently in countries of lower income' is a claim in the Harvard Consensus Statement on Antiretroviral Therapy for AIDS in Poor Countries[1] and reflects the perception of many in the developed world. Approximately one in every five sexually active adults in South Africa is currently infected with HIV.[2,3] The majority of these individuals will go on to develop AIDS and consequently die of the illness. Following the Durban 2000 AIDS Conference there has been increasing pressure from international organisations and activist groups for the pharmaceutical industry to reduce antiretroviral costs to the Third World. Drug prices have now decreased to such an extent that triple therapy has become an affordable and cost-effective option for an increased proportion of our population. An aspect that has received scant attention during the fight for cheaper therapy is precisely how these therapies will be delivered to larger segments of the public.

Triple therapy results in markedly decreased mortality for patients with advanced infection. It also reduces the number of opportunistic infections and hospitalisations.[3,4] Data from both South Africa and Brazil also demonstrate a reduction in tuberculosis infections in those taking antiretroviral treatment[5] (and personal communication with D Wilson, who will present data from a South African-treated cohort of patients at IAS, Buenos Aires, in July 2001). Despite these demonstrable advantages, therapy itself can be problematic. High pill burdens, complex regimens and adverse events make adherence to any antiretroviral regimen a challenging prospect for many.

The goal of antiretroviral therapy is to improve health by reduction of viral load to undetectable levels. This suppression allows recovery of immune function, reflected by an increase in CD4+ T cells, and stops disease progression. Near-perfect adherence is required to maintain viral load suppression, as is shown in Fig. 1.[6-8]

Poor adherence usually results in decreased viral suppression and may be associated with the development of drug-resistant virus. Resistant viral strains may be further transmitted to others.

Data from two major HIV drug trial sites demonstrate that

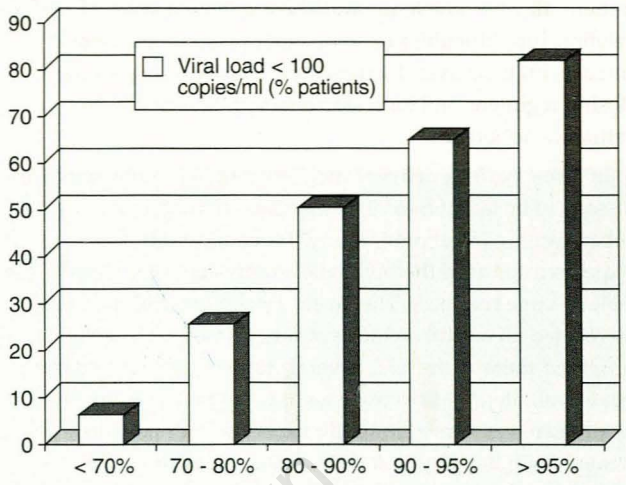

Fig. 1. Adherence with protease inhibitor therapy (Paterson et al., *Abstract 1-172, ICAAC, 1998).*

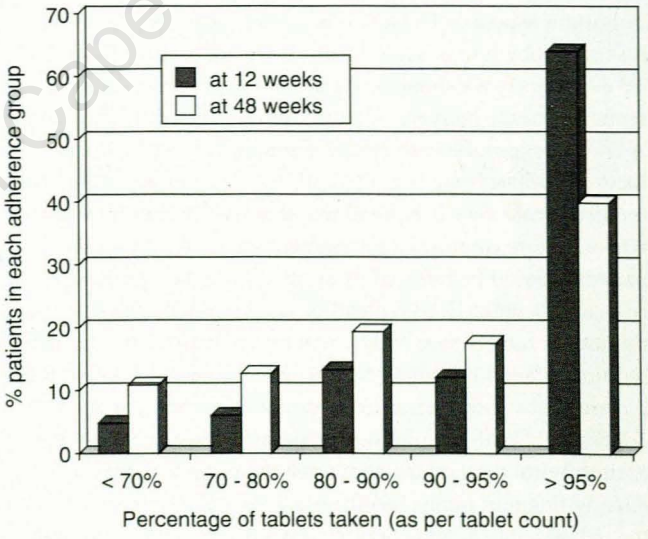

Fig. 2. Pattern of adherence to therapy at 12 and 48 weeks of treatment (Orrell C, Wood R. IAS Abstract, Buenos Aires, 2001).

high levels of adherence are achievable by South African patients. Adherence of a black cohort of patients to a variety of antiretroviral regimens (Fig. 2) was as good as or better than that attained in many other First-World countries (personal communication with C Orrell, who will present these data at IAS, Buenos Aires, in July).

The reasons for poor adherence can be complex, and often go beyond the doctor-patient relationship.[7] There are four key aspects of treatment adherence: the patient, the social milieu, the treatment regimen, and the programme support offered. Certain personality traits will make some patients more adherent than others, while even the most obsessive, careful

SOUTHERN AFRICAN HIV CLINICIANS SOCIETY

AIDS REVIEW

ANTIRETROVIRAL THERAPY IN SOUTH AFRICA — CAN WE DO IT?

As antiretroviral (ARV) drug prices plummeted in the last few months the question of accessibility for the public HIV-infected sector in South Africa became less 'can we afford it?' and much more 'can we do it?'. Approximately 4.8 million South Africans are currently infected with HIV, and the majority of them will go on to develop AIDS and consequently die of the illness[1] unless we can introduce ARV medications that are now increasingly available to the private sector to the public sector as well.

The logistical challenge of providing treatment on the scale required in South Africa, with other problems such as a relative paucity of resources, lack of expertise among treaters and the need to reach urban and rural populations, will demand innovative and radical thinking.

The World Health Organisation (WHO) recommends that, owing to the high cost of antiretroviral drugs, the complexity of the regimens and the need for careful monitoring, the following essential services and facilities must be in place before considering the introduction of antiretroviral therapy (ART) into any setting:

- Assured access to voluntary counselling and testing.
- Institution of follow-up counselling services for ART to ensure continued psychosocial support and to enhance adherence to treatment.
- Capacity to recognise and appropriately manage common HIV-related illnesses and opportunistic infections (OIs).
- Reliable laboratory monitoring services, including routine haematological and biochemical tests for the detection of drug toxicity as well as access to facilities for monitoring the immunological and virological parameters of HIV infection.
- Assurance of an adequate supply of quality drugs, including drugs for treatment of OIs and other HIV-related illnesses.
- Identification of sufficient resources to pay for treatments on a long-term basis.
- Information and training on safe and effective use of ART drugs for health professionals in a position to prescribe ART.
- Establishment of reliable regulatory mechanisms against misuse and misappropriation of drugs.

POLITICAL WILL

Before launching into any ideas of 'how to' a few words must be said about 'want to'. A nation-wide community-based ARV programme cannot exist without political will and support. Many issues such as the procurement of the cheapest therapies, provision of adequate resources and maintenance of international links with funding bodies and other organisations are all largely dependent on political backing. Countries such as Botswana and Uganda and some regions in South Africa have shown what can be achieved when political will exists. Botswana is exploring country-wide access to ART, Uganda has some early access projects, and the Western Cape has achieved 80% mother-to-child transmission (MTCT) prevention coverage of all pregnant women in the province and has an ART pilot project run by 'Medicine Sans Frontier' in Khayelitsha, with plans for more.[4]

COMMUNITY 'BUY-IN'

Recent media reports have begun to create the awareness of need in our HIV-infected population and there is growing dissatisfaction with the obvious and wide disparity between therapy in the First versus the Third World. MTCT prevention programmes have done much to sensitise whole communities to the HIV problem and lift some of the veils of secrecy and denial.

PARALLELS WITH TUBERCULOSIS CONTROL

The national tuberculosis programme necessitates the delivery and monitoring for a period of 6 months of a cocktail of drugs, including relatively hepatotoxic agents, to large numbers of patients. This is achieved by utilising scheduled therapy dispensed at primary health care clinics without routine laboratory safety monitoring, and an adherence strategy based on directly observed therapy (DOT).[3,4]

The logistics of the tuberculosis programme and a proposed HIV treatment programme are compared in Table I, and this comparison will serve as a discussion framework for this article.

Numbers

Following the recognition of long-term toxicities of ARV drugs, particularly protease inhibitors, international guidelines have become more conservative with respect to commencement of ART. Unlike tuberculosis, where a diagnosis of disease is followed by immediate treatment, there is a need to be more circumspect with respect to initiation of ART for HIV infection.

In developing countries where resources for treatment are more constrained than in the First World, we may need to limit

Table I . Comparison of TB and proposed ARV programmes

	Tuberculosis programme	Proposed HIV programme
Numbers treated in SA	Approx. 260 000 per annum	Approx. 200 000 per annum
Treatment period	6 months	Lifelong
Case identification	Passive case finding	Passive case finding
Diagnosis	Sputum AFB-positive	Clinical ± CD4 < 200/µl
Public health	Contact tracing	Treating family unit/partners
Treatment modalities	Schedules I and II (combination formulations)	Schedules ± modification (blister packs and combinations)
Drug supply	Inexpensive	High cost
Treatment compliance	DOTS	Adherence support
Monitoring	Sputum AFB	Clinical ± CD4 ± viral load
Setting	Primary health care	Primary health care

AFB = acid-fast bacilli.

ART initially to those symptomatic HIV-infected patients with AIDS who will derive maximal benefit from therapy.

Case identification

Patients will be identified as suitable for ART by passive case finding from voluntary counselling and testing sites (VTC), prevention of MTCT programmes, symptomatic HIV-infected patients presenting to primary health clinics, or patients co-infected with HIV and tuberculosis presenting to TB clinics. The diagnosis of AIDS is a clinical definition based on such manifestations as extrapulmonary TB, *Pneumocystis carinii* pneumonia, wasting syndrome, toxoplasmosis and cryptococcosis. When resources become more widely available, treatment could be expanded to include patients with WHO clinical stage 3 disease such as oral candidiasis or hairy leukoplakia, a CD4 T-cell count < 200/µl, or a total lymphocyte count < 1 250 cells/µl. This strategy would reduce the health care burden that these patients currently impose on in- and outpatient health resources.

Treatment modalities

The TB programme has been successfully run from primary health clinics on a large scale utilising two basic schedules that are simple and have recognised adverse effects. The scheduled approach simplifies management, training and drug supplies, ensures uniformity of medical care, and allows management by nurse practitioners and/or clinical assistants — a cadre of the health care profession much utilised in Africa.[5] Adherence data from Cape Town have shown that high levels of ART adherence can be achieved in patients from poor communities, and that regimens with twice rather than 3 times daily dosing and with drugs presented in blister packs were associated with better adherence. In addition, in designing schedules compatibility with pregnancy and/or tuberculosis medication will need to be considered. Other simple interventions such as provision of weekly pill boxes may also assist adherence.

Drug supply

Since an ART programme will involve high-cost drugs in high volume, there will need to be a secure delivery system. Should drugs be manufactured locally they will need to be shown to be biologically as well as chemically equivalent to imported ones. Finally, since the ARV drugs will be required for a long time and missed doses may result in viral resistance, there should be a reliable supply.

Adherence support

Compliance or adherence is a major obstacle to overcome in all ARV programmes worldwide. The Harvard Consensus Statement on antiretroviral therapy for AIDS in poor countries claims that 'patients will fail to take antiretroviral drugs consistently in countries of lower income',[6] and this reflects the perception of many in the First World. However, data from the HIV drug trial units in Cape Town and Johannesburg, and the MSF programme in Khayelitsha, demonstrate that adherence can be as good as or better than that attained in many First-World countries.[7] Aside from the treatment regimen itself, adherence is also very dependent on the individual support the ARV programme offers.[8] We have trained a group of HIV-infected peer counsellors in all aspects of ART including mechanisms of action, side-effect profiles, the rationale of treatment, and the need for good adherence. It is proposed that these therapeutic counsellors (TCs) act as a buffer between clinic staff and patients.The TC will help in the assessment of treatment readiness in the potential candidate and will run treatment readiness programmes in the patient's own language. Patients and TCs would set up alliances which would enable quicker reporting of serious side-effects and more careful surveillance of individual patients. In addition the use of cell phone technology to enhance communication is being investigated. In a study carried out in a corrective facility in the USA it has been shown that adherence to ART was much improved when therapy was delivered with DOT.[9] However,

while long-term DOT is practical in a corrective facility, the impact of lifelong ART on quality of life in patients leading busy normal lives needs to be assessed carefully.

HIV often affects more than one family member and adherence is also improved when family support is available.[10] Wherever possible treatment schedules should be similar for infected adults and children within the same family to assist with side-effect and adherence issues.

Monitoring

This aspect of ARV provision can be costly and has been cited as a reason for stalling implementation of ART on a wider scale. Monitoring includes measures of both safety and efficacy.

Safety monitoring. As far as possible the schedules chosen should comprise drugs that have few side-effects, and side-effects should be clinically monitorable or easily checked in the side-room, e.g. haemoglobin. Drugs that cause silent metabolic or hepatic inflammation, for example, may need to be avoided, while those to which patients manifest hypersensitivity with a rash or flu-like symptoms are easier to monitor on a large scale. The patient, TC and clinic staff must be well trained to pick up drug side-effects for the particular schedules being used and to differentiate them from opportunistic infections.

Efficacy monitoring. The First-World model has been that of quantitative viral load measurement with the goal of viral suppression. Advances in technology may result in significantly lower costs of viral quantification in the near future; however, in resource-constrained settings there will be a need to explore cheaper monitoring surrogates such as CD4 count, total lymphocyte count, or combinations of clinical and simple laboratory tests.

Clinical setting

If the goal of getting the large numbers of South Africans in need onto ART is to be realised, the programme cannot be run from large tertiary urban centers only, but will need an extensive primary clinic network. In the Western Cape, where the majority of the population live in the greater metropole area, strategic use of a few key existing primary health care facilities where training could be concentrated could incorporate a large proportion of the HIV-infected population. In other regions, where communities are more rural and distances greater, a wider network of primary health clinics will need to be utilised to ensure extensive coverage similar to that seen in the TB control programme.

Sustainability

In order to ensure sustainability of drug supply and resources for ART programmes, both international and national mobilisation of funding will be needed. International initiatives such as the Global Fund are one such option. It is also probable that for reasons of sustainability cheaper generic alternatives will need to be considered. Sustainability also requires that where possible the programme be integrated into the existing health infrastructure with adaptations to local needs. This will demand sufficient capacity building and training to ensure ongoing expertise.

Conclusion

Most importantly, for an ART programme to give hope to the 4.8 million HIV-infected citizens in South Africa, it will require bold political vision in terms of national AIDS policy and thinking. It will not be possible to introduce full-scale programmes across the country immediately. Yet there is tremendous urgency to begin. Where infrastructure already allows we should move forward immediately and have a systematic well-planned approach to extending the ART programme to include as much of the HIV-infected population as possible in the least possible time.

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Table VI. Logistics regression report

Response variable: Correct prediction			
Parameter estimation section			
Variable	**Regression coefficient**	**Standard error**	**Probability level**
Intercept	3.323	0.510	0.000
Days (in hospital)	0.005	0.010	0.602
Blunt (1 = blunt)	0.054	0.400	0.893
Age	–0.019	0.011	0.076
Hospital (1 = JH)	–0.463	0.333	0.164
Helicopter (1 = helicopter)	–1.300	0.400	0.001

JH = Johannesburg Hospital.

highly significant variable, only the patient's age is possibly significant ($P = 0.076$). Not surprisingly, the older the patient the less likely the TRISS methodology is to predict the probability of survival or death correctly.

CONCLUSION

Helicopters clearly deserve a place in the emergency care of trauma victims. However, this is only one link in the chain that will ultimately lead to either death or survival. For it to be successful, it must link reliable, efficient emergency medical services (road-based) and effective trauma centres that are staffed appropriately with a committed team of health care professionals. If used appropriately there appears to be little doubt that these expensive machines can play an important role in preventing certain unnecessary deaths while reducing costs for both individuals and health care facilities.[8,9]

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SPECIAL ARTICLE

EXPLORING THE COSTS OF A LIMITED PUBLIC SECTOR ANTIRETROVIRAL TREATMENT PROGRAMME IN SOUTH AFRICA

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Background. The role of antiretroviral treatment for adults in the pubic sector in South Africa is debated with little consideration of programme choices that could impact on the cost-effectiveness of the intervention. This study seeks to explore the impact of these programme choices at an individual level, as well as explore the total cost of a rationed national public sector antiretroviral treatment programme.

Methods. Eight scenarios were modelled of limited national treatment programmes over the next 5 years, reflecting different programme design choices. The individual cost-effectiveness of these scenarios were compared. The total costs of the most cost-effective scenario were calculated, and the potential for savings in other areas of health care utilisation was explored.

Results. The direct programme costs per life-year saved varied between scenarios from R5 923 to R11 829. All the costs of the most cost-effective scenario could potentially be offset depending on assumptions of health care access and utilisation. The total programme costs for the most cost-effective scenario in 2007 with 107 000 people on treatment are around R409 million.

Conclusion. Specific policy choices could almost double the number of people who could benefit from an investment in a limited national antiretroviral treatment programme. Such a programme is affordable within current resource constraints. The consideration of antiretroviral treatment calls for a unique public health approach to the rationing of health services in the public sector.

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Table I. Description of modelled scenarios and sensitivity analysis scenarios

The cost per life-year gained is presented at 5 years for the following eight intervention options:
(A) Baseline (generic medication, baseline testing and visit costs)
(B) Optional (higher proportion of doctor visits, and additional viral load testing)
(C) Patent medicine pricing scenario
(D) Second-line treatment offered to 50% of those with virological failure on first-line treatment at generic medicine prices
(E) Second-line treatment offered to 75% with virological failure on first-line treatment at generic medicine prices
(F) Patent medicine pricing and optional testing and consultation schedule (B & C combined)
(G) Patent medicine pricing, optional testing and consultation schedule, and 50% of those failing first line offered second-line treatment (B, C & D combined)
(H) Second-line treatment offered to 50% of those with virological failure on first-line treatment at generic medicine prices, with optional testing and consultation parameters (B & D combined)

In addition, sensitivity analyses are produced for the following seven changes in assumptions:
S1. (A) with no discount over time for medicines
S2. (A) with a 15% per annum discount over time for medicines for 3 years, followed by 7.5% annually
S3. (A) with no additional costs for each consultation compared to current services
S4. (A) with 2.5 times the current consultation costs for each consultation
S5. (A) with increased mean survival on first-line treatment to 3.6 years (from 2.68)
S6. (D) with increased mean survival on first line to 3.6 years and 7.41 years for consecutive regimens
S7. (A) with no provision for visits to the new services for people who are not on ARVs, and
S8. (D) with no discount over time for medicines

Whereas the role for antiretroviral therapy (ART) for HIV has been an issue for scientific and public debate in South Africa, a number of middle-income and poor countries have already initiated treatment programmes in spite of resource constraints (Brazil, Chile, Thailand, Nigeria, Senegal, Cote D'Ivoire). The recent publication by the World Health Organisation[1] of guidelines for the scaling up of antiretroviral treatment programmes is indicative of both a convergence of clinical thinking and the increasing pressure on health care systems to provide antiretroviral interventions.

In addition to the survival and quality of life benefits for individual patients,[2,3] many authors have pointed to the synergies between antiretroviral treatment programmes and preventive strategies.[4-7] Others have compellingly described the role extended HIV survival could have in reducing the burden on society and preserving our human and social infrastructure.[4,8]

The following model costs a rationed national antiretroviral treatment programme for adult South Africans that could conceivably begin in 2002. From the perspective of the public health system, we explore the relative cost-effectiveness of a number of ART-related policy options and the overall resource implications of a limited national antiretroviral treatment programme.

METHODS

Service model

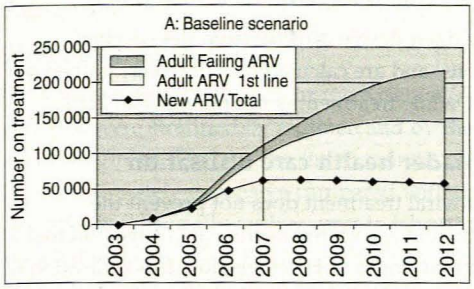
The approach to costing ART provision is based on an emerging service model in which:

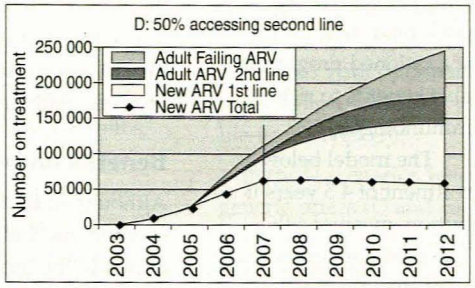
- Specific HIV/AIDS services are required to develop the relationships between patients and clinicians, to ensure continuity of care, and to provide a mechanism through which patients can be evaluated for potential enrollment onto an ART programme.
- Consequent on meeting predetermined eligibility criteria, which are a combination of clinical and (possibly) social assessment,[9] patients are considered eligible for ART around the time that they become AIDS symptomatic.
- After commencing treatment, patients are managed through the HIV/AIDS service, but still attend regular services for other routine and acute care.

Numbers on treatment

A spreadsheet model with eight scenarios (Table I) was used to anticipate the numbers of people on antiretroviral treatment over the next 10 years. In the model, the number of new patients receiving treatment was gradually increased over 5 years. The cumulative number of people surviving on treatment in the model by the middle of 2007 varied between scenarios from 106 911 to 117 621, depending on survival assumptions and whether or not second-line treatment was offered to a proportion of those failing the first-line regimen (Fig. 1).

Those on treatment were stratified into a number of subgroups, reflecting those on a first-line regimen, those on a second-line regimen, and those failing treatment. The first 6 months of a new regimen were distinguished from the remaining time on the regimen. The model was run for a





Scenario	Mid year	2003	2004	2005	2006	2007
	A: Baseline	3 130	10 403	27 301	59 812	106 911
	D: 50% accessing second line	3 179	10 586	27 930	61 420	110 361
	E: 75% accessing second line	3 204	10 677	28 249	62 254	112 181
	S5: A, with median survival 4 years on first line	3 211	10 836	28 691	63 367	114 491
	S6: D, with median survivals 4 and 6.5 years	3 253	10 982	29 224	64 782	117 621

Fig. 1. Numbers on treatment by category of treatment in modelled scenarios.

further 5 years with constant assumptions in order to explore the medium-term impact of deferring costs. By 2007 the numbers of people accessing treatment in the model could represent 10% of those becoming AIDS-symptomatic that year (compared with estimates of adult deaths in the subsequent 2 years[10]). The combined impact on new infections of extended survival, reduced viral load and altered behaviour for those on treatment was assumed to be neutral compared with a no-treatment scenario. Additional or averted new infections are likely to have little impact on the total direct treatment costs in the timeframe of this analysis.

Direct programme costs

Medicines

The biggest cost-driver of ART is undoubtedly the medicine costs. In this model, the limited number of medicines selected is sufficient for a single regimen or for two independent regimens, while still sufficiently restricted to limit costs in both the generic and patent pricing scenarios. It is assumed that all rationally selected starting regimens have equivalent treatment outcomes.[11]

The estimated proportion of patients likely to switch from any single medicine due to intolerance is based on the experience to date in Khayelitsha[12] validated by HIV clinicians. In cases of single-medicine changes, the first 6 months of treatment are apportioned to the starting medicine, while the remaining time on the regimen is apportioned to the medicine to which the patient changes. In effect virological failure and intolerance are modelled separately, with the crude assumption that 6 months is the average time at which a medicine is changed for reasons of intolerance. It was necessary to include intolerance-driven individual medicine switches within first- and second-line regimens as they impact significantly on overall medicine costs in the baseline scenario (where generic pricing is utilised). Where a combination tablet could substitute for individual medicines, the price of the combination tablet was included.

Future price changes are another important variable in anticipating medicine costs. The model presented assumes an annual price reduction in real terms of 10% per year for the first 3 years and 5% annually thereafter. Sensitivity analysis explores the impact of no reduction in price and a 15% initial reduction (for the first 3 years) followed by 7.5% per annum.

Laboratory monitoring

Based on the WHO recommendations,[1] the model incorporates two testing scenarios. The first provides for all tests (including a twice-yearly CD4 count and a CD4 count before enrolment) except for viral load testing. The second is an optional scenario in which viral load tests and CD4 counts are conducted three times a year.[13]

Visit costs for the antiretroviral treatment programme

Allowing that consultation costs (including additional staff training) may be higher than for standard primary care consultations, a factor of 1.5 is applied to the average cost of a primary care consultation in the Western Cape metropole (where there are doctor- and nurse-driven services). This factor is varied between 1 and 2.5 in the sensitivity analysis.

The visit schedule applied is that used in the current Medicin Sans Frontieres treatment protocol[9] to estimate the additional visits required as a result of ART. Again two scenarios are built; one in which a proportion of the visits are at a lower cost structure as the visit is principally to ensure adherence and dispense medicines, and another where the majority of the visits are with a doctor.

Provision is also made for visits to HIV/AIDS services by a proportion of HIV-infected people in WHO clinical stage 3. This proportion is set equal to the proportion of patients accessing treatment when becoming AIDS-symptomatic. The model assumes an average of 3 visits per year for those not on antiretrovirals but attending the HIV/AIDS services as a prelude to possible enrolment in an ART programme. These visits are costed at the existing clinic consultation costs, and form a substantial component of the workload.

Anticipating the individual benefits of treatment

The survival benefits of ART are not yet fully described. Published data at 3 years since the initiation of therapy, however, suggest a remarkable reduction in the anticipated

mortality.[2,3] UNAIDS currently recommend that a survival benefit of 5 - 7 years be used for HIV modelling in rich countries.[14] Early indications suggest that a rationed programme in South Africa is likely to have comparable benefits to rich countries, taking into account baseline immunological characteristics at the onset of treatment.[15,16] The model below uses a median survival from initiating treatment of 4.5 years if two regimens are offered (weibull distribution, mean of 6.06 years of which the *benefit* is 4.46 years). The model assumes that treatment is failing for a period at the end of treatment of equivalent duration to WHO stage 4. It is further assumed that, of the survival benefit, 60% is derived from the first regimen, and the remainder from an alternative regimen. For the scenarios in which second-line treatment is included, it is assumed that not everyone in whom a first regimen fails will be offered a second regimen, either because they have exhausted their affordable treatment options through intolerance-driven individual medicine changes, or because they do not meet additional eligibility criteria for second-line treatment.

The life-years gained derive directly from the survival benefit assumptions, and are calculated as life-years gained per year on treatment while treatment is not failing (Fig. 2).

Benefits on broader health care utilisation

Although antiretroviral treatment does not prevent the eventual morbidity and associated health care utilisation that occurs in the terminal stages of HIV infection, this utilisation is deferred by the duration of the survival benefit. Within the realistic timeframes of any planning exercise of this nature, this deferment could result in a real benefit to the health care system. The key contributors to the cost saving are the principal cost drivers of hospital inpatient days, ambulatory consultations and tuberculosis treatment. No discounting was applied to deferred costs.

The estimates used (Table II) are similar to those used in other HIV costing studies.[17,18]

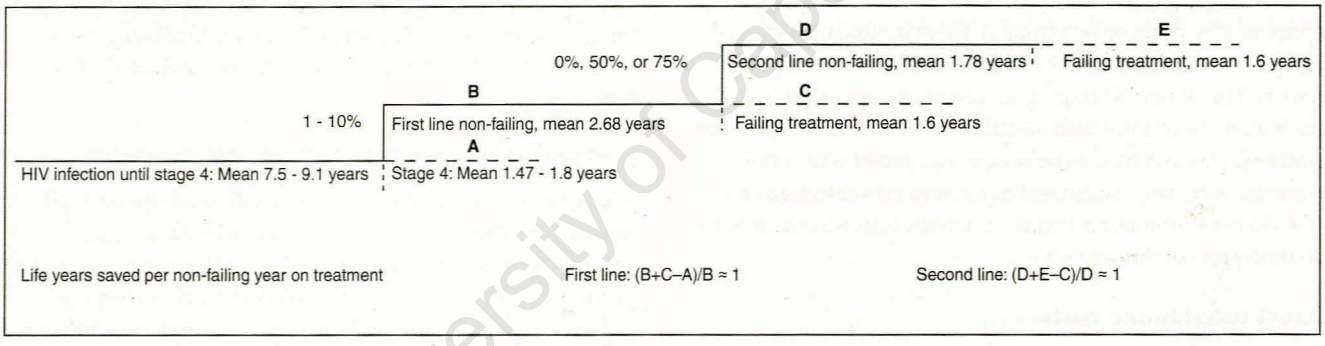


Fig. 2. Survival and effectiveness assumptions.

Table II. Cost and utilisation assumptions

		Utilisation				Cost		
	Units	Stage 4	ARV FL	ARV SL	Failing	Units	Min.	Max.
Non-ARV HIV-related clinical costs								
Hospitalisation (1)	Inpatient days	18.8	2.8	3.8	18.8	Inpatient day	R530	R530
Clinic consultations (2)	Extra consults	11	2	2	11	Visit cost	R73	R73
Tuberculosis (3)	Annual risk	36%	4%	4%	36%	Treatment cost	R1 560	R1 560
ARV-related costs								
ARV medicine costs (4)	ARV utilisation		100%	100%	50%	Annual cost	R4 612	R15 288
ARV laboratory costs (5)	Extra tests		Yes	Yes	No	Annual cost	R457	R2 206
ARV visit costs (6)	Visits		12	12	3	Annual cost	R821	R986

(1 & 2) Utilisation assumptions the same as used in the study by Abt and associates,[17] with a reduction in length of stay from 10 days to 8 days for stage 4 based on more recent inpatient studies. Clinic visits are calculated at the current price per consultation in primary health care services in Cape Town, and reflect excess visits as a result of HIV. Utilisation while on ARVs is premised on an 80% reduction in hospitalisation and clinic visits compared with stage 4.
(3) Taken from cohort data in Cape Town,[21] with additional assumption of relative risk of tuberculosis between stages 4 and 3 of 2, and relative prevalence between stages 3 and 4 of 2. Cost per completed treatment from Western Cape Department of Health, reflecting only a proportion (60%) of the DOTS-related costs so as not to double-count the tuberculosis-related PHC, medicine and hospitalisation costs.
(4) Starting regimen in generic scenarios is Triomune (D4T, 3TC, NVP, $295/year), with 57% still on Triomune by 6 months due to intolerance-driven individual medicine switches. Second-line regimen for generic scenarios is AZT, DDI and INV/RTV. The same regimen is the cheapest patented regimen as well (used here as individual drugs) unless DDI and D4T are combined. Medicine costs calculated for on average half of the time after treatment has started failing. It is hoped that LPV/RTV could replace INV/RTV with little impact on costs if preferential pricing is obtained.
(5) Range of tests depending on individual medicines include FBC, diff., creatinine, ALT, cholesterol, glucose and amylase. Twice-yearly CD4 counts (R83 per test) are included additionally in pragmatic scenarios. Thrice-yearly viral loads (R550 per test) and CD4 counts are included only in the 'optional' scenarios.
(6) 80% of visits in the 'optional' scenarios, and 60% in the remaining scenarios, are with a doctor. The non-doctor visits are with a nurse and/or counsellor, and are principally to support adherence and dispense medicines. Doctor visits are calculated at the current price per consultation in primary health care services in Cape Town, with an additional cost of 50% varied to an additional 150% in sensitivity analysis. Non-doctor visits are costed at 75% of the reference costs.

RESULTS

Direct treatment costs

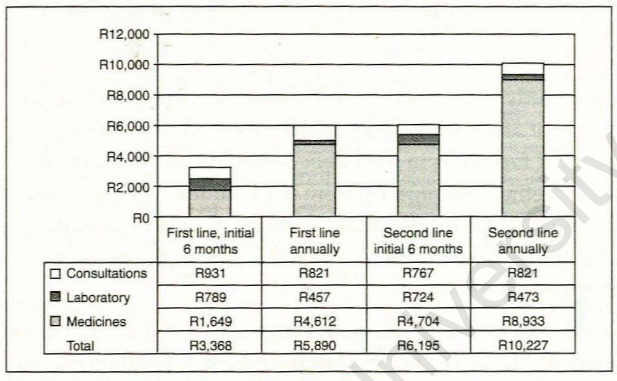
The direct costs of treatment for the baseline (A and D) scenarios were stratified by regimen and by the first 6 months on each regimen (Fig. 3). The first 6 months of treatment are disproportionately expensive compared with the subsequent annual costs, especially with respect to laboratory and consultation costs. The differential between first-line and second-line medicine prices when accessing generic medicines is clearly evident. The costs per treatment year (after the first 6 months) vary between scenarios and regimens from R5 890 (A: first-line) to R15 288 (G: second-line). Medicine costs dominate expenditure across all scenarios and regimens.

Cost per life-year gained

The use of generic versus patented medicines is the single most important factor impacting on the costs per life-year gained at 5 years (Table III). The cost per life-year gained is 48 - 53% greater when patented medicine prices are utilised. Additional testing and switching most of the consultations to doctors increased costs by a further 45%. Combining patented medicines with optional laboratory monitoring and consultation schedules yielded a 99% increase in the cost per life-year saved (F).

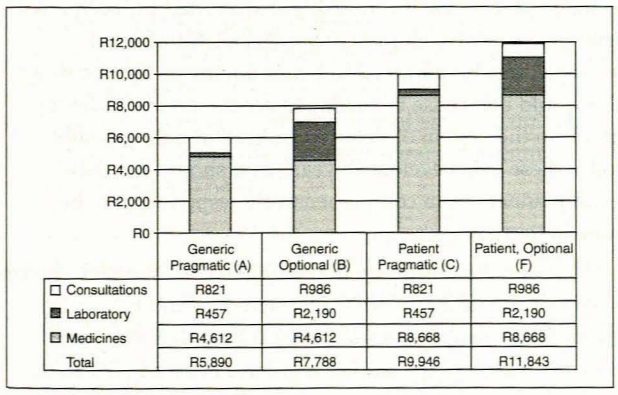
In those scenarios where second-line treatment is included at generic prices (D and E), the marginal cost per life-year saved when adding this treatment is 36 - 39% higher than the baseline cost (A: first-line only).

Sensitivity analysis reinforced the pivotal role of assumed changes in future medicine pricing (S1 and S2: 23% increase in the baseline cost per life-year saved over 5 years if no price reductions). It also demonstrated the relatively small impact of changing assumptions on the services required before enrolment (S7), the cost-structure for consultations (S3 and S4), or the duration of the survival benefit on treatment (S5 and S6).



	First line, initial 6 months	First line annually	Second line initial 6 months	Second line annually
Consultations	R931	R821	R767	R821
Laboratory	R789	R457	R724	R473
Medicines	R1,649	R4,612	R4,704	R8,933
Total	R3,368	R5,890	R6,195	R10,227

Changing costs for baseline (A) scenario based on regimen and duration on regimen, 2002 prices



	Generic Pragmatic (A)	Generic Optional (B)	Patient Pragmatic (C)	Patient, Optional (F)
Consultations	R821	R986	R821	R986
Laboratory	R457	R2,190	R457	R2,190
Medicines	R4,612	R4,612	R8,668	R8,668
Total	R5,890	R7,788	R9,946	R11,843

Differential costs between scenarios, for the first-line regimen following the initial 6 months of treatment, 2002 prices

Fig. 3. Direct costs at an individual level for antiretroviral interventions.

Total costs and potential resource savings as a result of deferred treatment

In the most cost-effective scenario, the total direct costs of a programme of this size are estimated to be R409 million in the year 2006 - 2007. Taking into account the deferred hospitalisation and consultation costs for those on ART, there is a considerable impact on resource utilisation. When quantified financially over 5 years, this covers the cost of antiretroviral treatment (135% of direct programme costs averted). At 10 years some of the deferred costs have re-entered the system, reflected by a reduction in cumulative savings as a percentage of the direct intervention costs over this period (90% of intervention costs).

DISCUSSION

This exercise illustrates how policy choices impact on the benefits of a rationed ART programme and how those benefits are distributed. It is immediately apparent that accessing cheaper medicines could significantly extend the impact of such an intervention. We used generic pricing in our baseline scenario based on the sincere belief that it is a realistic policy option which has been successfully applied in a number of countries (generics of zidovudine and lamivudine have recently been registered by the Medicines Control Council, although they are still inaccessible due to patent restrictions). It is sometimes argued that medicine costs are over-emphasised. This analysis supports a strong emphasis on medicine costs.

While the cost of monitoring patients on ART has fallen over the past year, some investigations (viral load testing in this analysis) remain prohibitively expensive for the benefit they add, and should not necessarily be an automatic component of a public sector programme until their costs are reduced.

The marginal cost per life-year saved when adding second-line treatment to the baseline scenario was considerably higher

Table III. Cost-effectiveness and sensitivity analyses

	Cost/life-year	% change from baseline	Marginal cost/life-year	% change from first line
A: Baseline	R5 923			
B: Optional	R8 595	45		
C: Patent pricing	R9 089	53		
D: 50% second-line access	R6 082	3	R8 215	39 (1)
E : 75% second-line access	R6 136	4	R8 042	36 (2)
F: B & C combined	R11 761	99		
G: B, C & D combined	R11 829	100	R12 736	8 (3)
H: B & D combined	R8 775	48	R11 202	23 (4)
Sensitivity analysis				
S1: A with no medicine discount	R7 266	23		
S2: A with medicine discounts 50% higher	R5 388	–9		
S3: A with no additional cost for consultations	R5 571	–6		
S4: A with visit cost factor 2.5	R6 627	12		
S5: A with first-line survival 4 years	R5 675	–4		
S6: D with combined survival of 6.5 years	R5 797	–2	R8 184	44 (5)
S7: A with no non-ARV visit costs	R5 398	–9		
S8: D with no medicine discounts	R7 528	27	R11 048	52 (6)

(1 & 2) compared to A, (3) compared to F, (4) compared to B, (5) compared to S5, (6) compared to S1.

(39%) than the cost without this option. This increase is partially masked by the reductions in medicine prices over time, as those accessing second-line treatment are doing so a few years into the programme. One sensitivity analysis (S8) demonstrates that the marginal increase could be as high as 52% without the effect of the medicine price reductions. Although clinicians baulk at limiting ART to a single tier of therapy, the option of spreading a smaller benefit to a greater number of patients needs serious consideration, bearing in mind the additional health system implications of enrolling additional new patients. We are not necessarily advocating that only one tier of therapy be considered. A gradual implementation of ART would have relatively few people on second-line treatment by 5 years, with the total programme costs not being considerably more expensive than the baseline programme cost presented. It is also likely that the differential cost between first- and second-line treatment will reduce over time as a greater number of antiretrovirals are produced by generic manufacturers. If the price differential remains large and financial resource constraints are considered the limiting factor to more widespread treatment, and if maximal diffusion of treatment is a policy goal, then an examination of the marginal costs of second-line treatment will remain important.

Even without offsets, the total costs of a programme of this size are a small fraction of anticipated health sector expenditure on HIV/AIDS. Although many may find the size of this programme unpalatably small, a programme that is approaching 100 000 people on treatment within 5 years would be a significant achievement.

This study has a number of limitations. Importantly, in the absence of an existing policy to provide antiretrovirals as treatment for adults in the public sector, it was necessary to piece together assumptions from many different sources. Some assumptions should be treated with great caution, in particular those anticipating health service utilisation in the absence of antiretroviral treatment.

Although estimates of health care utilisation that derive from cohort studies may not apply to the whole population due to unequal access, it is likely that those who do access the ART programme would have been able to access clinical services had they not received the intervention. Given the huge excess demand for services, it is unlikely that averted utilisation will result in financial savings in any but the least-affected provinces, resulting instead in better quality of care for those who would otherwise be unable to access services. The re-entry into the system of deferred utilisation only partially erodes these gains even at 10 years, and should not deter health planners from comprehensively responding to the immediate crisis.

It has been demonstrated that with the correct policy choices, the cost of providing an ART intervention could be considerably cheaper per year of treatment than has been quoted by many of the studies that have deemed ART to be unaffordable.[17,19,20] It has been further demonstrated that the averted costs could result in savings that make the intervention cost-saving, or at least significantly more cost-effective than an examination of the direct costs yields. This is only from the perspective of the health sector without consideration of

possible synergistic prevention gains. Even with the averted costs factored in, when the modelled intervention is scaled up to cover much greater percentages of those in need, it demands extraordinary expenditures. How can a cost-saving intervention be unaffordable?

The public health system is implicitly rationing services through reduced access to care, and the extent of this is likely to increase. A modelled ART programme that assumes more extensive access than is implicitly provided for non-ART services at present, will appear unaffordable even if it is cost-saving at an individual level.

CONCLUSIONS

There are very clear policy choices, political and clinical, which for the same expenditure could double the number of people benefitting from a rationed ART programme. A programme that utilises generic medicines, is pragmatic with respect to laboratory monitoring and consultations, and maximises the diffusion of benefits, is the most cost-effective, and is considerably cheaper than many previous estimates suggest.

Whereas we should strive to provide treatment to as many of those in need as possible through the future mobilisation of additional resources and campaigning for price reductions in medicines and laboratory tests, a rationed treatment programme *is currently affordable* within existing resource constraints, and would have enormous benefits. We should not make our provision of this intervention consequent on raising additional resources, and the decision to proceed could in fact aid our attempts to mobilise external financial resources. The public good resulting from the broader impact on prevention and morale, which is arguably one of the major benefits of introducing a rationed programme now, could be substantially realised with relatively small numbers on treatment.

The present and anticipated HIV burden on the public health care system is such that rationing is inevitable, with the prospect of planning for a new intervention such as ART requiring us to be explicit about our inability to meet demand. This paper serves to highlight the need for a revised public health discourse around rationing to deal with the unique challenges faced in providing antiretroviral interventions where they are most needed.

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Adherence is not a barrier to successful antiretroviral therapy in South Africa

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Objective: to determine adherence of an indigent African HIV-infected cohort initiating antiretroviral therapy (ART); to identify predictors of incomplete adherence (< 95%) and virologic failure (> 400 HIV RNA copies/ml).

Design: Prospective monitoring of adherence in a poor HIV-positive cohort, attending a public sector hospital and receiving ART through phase III studies.

Methods: Adherence to ART was determined over 48 weeks by counting tablet-returns. Logistic regression models including age, WHO HIV stage, home language, socio-economic status, complexity and type of regimen were fitted to determine predictors of incomplete adherence and virologic failure at 48 weeks.

Results: 289 patients were recruited between January 1996 and May 2001. Median (mean) adherence of the cohort was 93.5% (87.2%). Three times daily dosing [risk ratio (RR), 3.07; 95% confidence interval (CI), 1.40–6.74], speaking English (RR, 0.41; 95% CI, 0.21–0.80) and age (RR, 0.97; 95% CI, 0.94–0.99) were independent predictors of incomplete adherence. Socio-economic status, sex and HIV stage did not predict adherence. Independent predictors of virologic failure included baseline viral load (RR, 2.57; 95% CI, 1.57–4.22) and three times daily dosing (RR, 2.64; 95% CI, 1.23–5.66), incomplete adherence (RR, 1.92; 95% CI, 1.10–3.57), age (RR, 0.96; 95% CI, 0.92–0.99) and dual nucleoside therapy (RR, 2.69; 95% CI, 1.17–6.15).

Conclusion: The proportion of individuals achieving viral suppression matched results from the developing world. Speaking the same language as site staff and simplified dosing frequency were beneficial. Socio-economic status had no impact on adherence and should not be used as a limitation to ART access.

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Keywords: antiretroviral therapy, Africa, adherence, viral load, socio-economic status

Introduction

The majority of HIV infected individuals live in sub-Saharan Africa. Over 4.7 million people are infected in South Africa alone, approximately one in every five adults [1,2]. South Africa is a middle-income, developing country and until recently, treatment with antiretroviral therapy (ART) on a large scale was considered financially impossible. In 2001, however, the local pricing of triple ART decreased by approximately 75%, and it is now available for less than $1000 per year. A recent out of court settlement between the government and pharmaceutical industry has further encouraged reduced antiretroviral pricing, which will expand access to South Africans receiving health care in the public sector.

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Expectation of poor adherence is a major concern in expanding therapy to South Africans, many of whom live in severe poverty [3,4]. A spokesman for USAID encapsulated these assumptions with his statement: 'Ask Africans to take their drugs at a certain time of day, and they do not know what you are talking about' [5]. This is concerning because adherence to therapy is a strong predictor of viral load suppression, immune recovery, disease progression, and death [6–13]. There is also the widely held belief that non-adherence to therapy among sub-Saharan Africans will lead to rapid development and spread of HIV drug resistance [3]. In contrast to current expectations of non-adherence, Laurent *et al.* reported high levels of adherence in Senegal [14.] As yet there are no published studies of objectively measured adherence in resource-limited settings comprising the majority of HIV infected patients.

We set out to measure pill count adherence and virologic suppression in a cohort of semi-urban South Africans living in extreme poverty.

Methods

Eligibility and recruitment

Participants were recruited from the Cape Town AIDS Cohort (CTAC), a group of HIV-positive individuals presenting to University of Cape Town HIV clinics which serve largely indigent populations. Patients were referred to the clinic study site between January 1996 and May 2001 by a broad spectrum of health care workers in the public sector of the wider Cape Town area. All participants were antiretroviral naive and provided written consent to participate in multi-centre phase III clinical trials of combination ART. These studies were approved by the University of Cape Town Research Ethics Committee. Inclusion and exclusion criterion were trial determined. All antiretroviral naive patients who commenced ART on any established study by December 2000 were eligible for adherence monitoring.

ART, routine medical care from the site doctors and nursing staff, and trial-related laboratory monitoring were free. The routine public service hospital system, which requires payment from the patients proportional to their income, was accessed for treatment of intercurrent problems, opportunistic infections or non-trial medication.

A single group education session (usually in English) is held prior to the consenting process and inclusion onto a study. There is no dedicated adherence counseling service, structured adherence support or formal adherence intervention as part of treatment. Patients access the site for 1–2 h every 2 or 3 months. There are no off-site visits by health care staff.

Demographic and socio-economic data collection

The socio-economic status of participants was assessed using the Cape Metropolitan Council per-suburb composite index which is based on household income (percentage of households earning less than US $1500 per annum), education level (percentage of adults with less than 8 years of schooling), unemployment status (unemployed adults who are actively seeking work as a percentage of all adults), welfare status (percentage of household heads who are single women with three or more children) and overcrowding status (percentage of households with more than 1.5 people per habitable room). The composite index is the sum of these five percentages (maximum 500%) and tends to range from 4 to >70. A score of >28.5 correlates well (r, 0.7) with extremely poor conditions of living (shack or informal dwellings with outside tap or public sector housing blocks). An index of 10–28.5 indicates a moderate standard of living (low-cost housing, basic amenities), and an index of <10 indicates a high standard of living (brick house, full amenities) [15]. For analysis purposes a cut-off of 28.5 was used as the break between poor and reasonable socio-economic circumstances.

Other demographic variables recorded include: age at commencement of therapy, language spoken at home (to question the impact of English-based education processes on adherence), sex, World Health Organisation (WHO) HIV stage, as well as viral load and CD4 cell count at baseline.

Antiretroviral treatment

Patients were assigned to one of six multi-center phase III studies. Patients in two studies in 1996 were given dual therapy with an additional third concurrent, placebo-controlled and double-blinded drug (placebo versus a non-nuceoside reverse transcriptase inhibitor regimen). In the other four studies patients were given triple therapy regimens.

Adherence monitoring

Adherence to therapy was assessed using clinic-based pill counts and pharmacy refill data over a period of 48 weeks. All ART was dispensed from the single study site. Patients were provided with more medication than required, i.e. tablets were usually dispensed in multiples of 30, whereas visits were booked in multiples of 28 days. Patients were instructed to return all medication bottles and unused pills at each study visit, but were not told that the returns were to be counted. All tablets of each antiretroviral medication were counted prior to dispensing and upon return. Adherence to therapy was calculated using the formula: (sum of tablets dis-

pensed – sum of tablets returned) / (total tablets prescribed over the 48-week study interval).

Patients who did not complete at least 30 days of ART were excluded from the analysis. Patients who completed at least 30 days of therapy, but who failed to bring any tablet returns over the study period have been included in the analysis and assigned a 0% adherence value. For those who withdrew from the study before 48 weeks, the last data available has been brought forward to be included in the 48-week data analysis.

Statistical analysis

Chi-squared test was used to compare categorical data and the student's t test was used for continuous variables. Associations were examined at the $P < 0.05$ level of significance. Logistic regression models were fitted to determine variables predictive of adherence and virologic failure. Only variables which were predictive on univariate analysis ($P < 0.05$) were included in the multivariate modeling.

Factors used in the univariate model for predicting adherence less than 95% included age (modeled as a continuous variable), language spoken at home (English), three times daily medication dosing, socio-economic status (low), WHO HIV clinical stage (stage 3 or 4), sex (female), and baseline CD4 cell count and viral load.

The univariate models for virologic failure (> 400 HIV RNA copies/ml at 48 weeks) included the baseline viral load, age, daily dosing schedule, adherence and antiretroviral regimen type. Viral load at baseline, age, three times a day regimens, medication-related food restrictions, taking more than 10 tablets a day, adherence < 95% and dual nucleoside therapy were modeled as baseline risk factors.

Results

Recruitment and retention

A total of 289 patients was eligible for adherence monitoring. Eleven patients (3.8%) were excluded from the analysis because they withdrew consent (six patients) or discontinued therapy due to toxicity (five patients) within 4 weeks of treatment initiation. An additional 36 patients (12.4%) withdrew from the study after the first 4 weeks and were included in the analysis.

Patient and regimen characteristics

The mean age of the cohort at commencement of ART was 33.4 years (SD, 8.7) and 43% of the cohort was female. Forty-two per cent of the cohort was drawn from poor socio-economic conditions areas (approximately US $1500 per annum per household). A further 20% came from moderate income areas (approximately US $5500 per annum per household). Only 20% of the cohort spoke English as their home language. The majority of the cohort spoke Xhosa, the local African language (48%), or Afrikaans (28%). Demographic and clinical characteristics of the cohort are presented in Table 1.

Regimens containing protease inhibitors were used by 120 (41.5%) patients. Ninety-four (32.5%) patients received non-nucleoside based regimens, 30 (10.4%) took triple nucleoside regimens and 45 (15.6%) of the patients, who commenced treatment in 1996, received dual nucleoside reverse transcriptase inhibitor therapy. Fifty-five percent of the cohort took more than 10 tablets a day and 41% had dietary restrictions related to antiretroviral medication.

Treatment discontinuation

Forty-seven patients (16.2%) discontinued therapy within 48 weeks. Those who discontinued were significantly younger, and had increased viral loads at baseline, with lower CD4 cell counts. Socio-economic status, sex, language spoken at home and WHO stage of HIV illness were not associated with discontinuation.

Treatment adherence and viral suppression

The median adherence of the cohort up to 48 weeks was 93.5% (mean, 87.2%; n, 278). This includes eight patients who did not return any tablets after continuing therapy beyond 4 weeks and who were assigned 0% adherence. Sixty-three percent of the patients main-

Table 1. Baseline characteristics of the cohort (n, 289): patients who completed 48 weeks of therapy were compared with those who withdrew before 48 weeks. Chi-squared analysis for categorical data and t tests for continuous data show that there was no significant difference in the groups with regards to sex, socio-economic status, HIV stage and home language. The group withdrew were significantly younger, and had increased viral loads with decreased CD4 cell counts at baseline.

Characteristic	Completed [n (%)]	Discontinued [n (%)]	*P*
Total number of patients	242	47	
Mean age [years (SD)]	34.1 (8.4)	31 (8.6)	$P < 0.05$[a]
Female	105 (43.4)	19 (40.4)	$P = 0.7$
Home language (English)	47 (19.4)	10 (21.2)	$P = 0.8$
WHO stage (3 or 4)	119 (49.2)	18 (38.3)	$P = 0.2$
Low socio-economic status	103 (43.6)	17 (36.2)	$P = 0.4$
Mean CD4 cell count (SD)	268 (165.2)	197 (147.8)	$P < 0.01$[a]
Mean $\log_{10}$ viral load (SD)	5.49 (0.67)	5.71 (0.75)	$P < 0.05$*

[a]t test for age, viral load, CD4 cell count), χ^2 test for the remaining variables.

tained adherence of ⩾ 90% to the prescribed tablets. There was no significant difference in adherence to protease regimens compared to non-nucleoside based regimens. The proportion of subjects in each adherence category is illustrated in Fig. 1.

Dosing interval, age and speaking English at home were independently associated with incomplete adherence (Table 2). Medication-related food restrictions (RR, 1.01; 95% CI, 0.63–1.63), taking 10 or more tablets a day (RR, 1.23; 95% CI, 0.77–1.98), low socio-economic status (RR, 1.42; 95% CI, 0.88–2.29) and HIV stage 3 or 4 (RR, 0.90; 95% CI, 0.56–1.45) were not significantly associated with adherence in univariate models and therefore were not included in the multivariate model.

Adherence, modeled as a continuous variable, was significantly associated with reduction in viral load at 48 weeks (r, −0.2923; $P = 0.001$). Of those who reached 48 weeks of therapy (n, 242), 66.1% had a viral load of < 400 HIV RNA copies/ml. This included 70.9% of those on triple therapy and 41.0% of those on dual therapy. For those who were ⩾ 95% adherent at 48 weeks, 73.4% had a viral load of < 400 copies/ml compared with only 61.0% of those whose adherence was < 95% ($P = 0.018$).

Independent predictors of virologic failure included baseline viral load, three times daily dosing, adherence < 95%, age and dual nucleoside therapy. Taking 10 or more tablets a day (RR, 1.11; 95% CI, 0.57–2.17) and medication-related food restrictions (RR, 1.95; 95% CI, 0.95–4.03) were not independently associated with virologic failure at 48 weeks (Table 3).

Discussion

We found that indigent patients recruited from the greater Cape Town area, receiving free therapy on clinical trials, took 93.5% of their medication as measured by clinic-based pill count and, of those on triple therapy, 70.9% maintained a viral load of < 400 copies at 1 year in the absence of formal adherence intervention. This data is consistent with the high levels of self-reported adherence and viral suppression in poor patients receiving ART in Senegal [14]. This level of adherence and viral suppression is similar to or better than that reported in most observational and clinical trial cohorts in developed countries where objective measures of adherence indicate that patients take 70% of their HIV antiretroviral medications (range 53–93%) and rate of viral load suppression is 50% (range 37–72%) on similar regimens [6–10,16–18].

Living in poor socio-economic circumstances, as defined by the Cape Metropolitan Council index, did not impact on adherence to therapy [15]. In contrast to our study, most patients receiving ART in sub-Saharan Africa directly purchase their medications and pay for

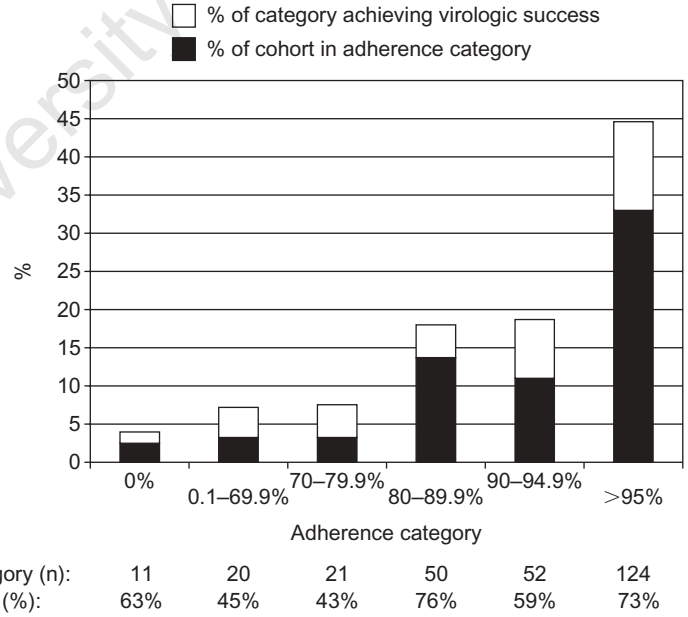

Fig. 1. Proportion of patients in each adherence category at 48 weeks (n, 242). Each full column represents the percentage of the cohort (n, 278) in each adherence category. The actual numbers per category are given below the graph. The 11 people with no tablet returns are recorded as 0% adherence. The dark shaded area within each column reflects the proportion of patients in that adherence category who achieved an undetectable viral load at 48 weeks. The actual percentage that achieved virologic success is recorded below the graph.

Table 2. Three times daily dosing predicted adherence < 95% in both the univariate and multivariate analyses. Increasing age and speaking English as a home language were protective against poor adherence in both analyses. Socio-economic index, sex, number of tablets per day, medication-related food restrictions and stage of HIV disease did not impact on adherence.

	Univariate analysis		Multivariate analysis	
Variables	RR	95% CI	RR	95% CI
Age	0.97	0.94–0.99	0.97	0.94–0.99
Home language (English)	0.45	0.25–0.84	0.41	0.21–0.80
Dosing (three times a day)	2.07	1.11–3.84	3.07	1.40–6.74

RR, Risk ratio; CI, confidence interval.

Table 3. In the univariate analysis baseline viral load, three times daily dosing, dual nucleoside regimens, taking more than 10 tablets a day and medication-related food restrictions predicted virological failure (> 400 HIV RNA copies/ml) at 48 weeks. Increasing age and adherence > 95% were protective against failure. In the multivariate model baseline viral load, three times daily dosing and adherence < 95% predicted virological failure. Increasing age remained protective.

	Univariate analysis		Multivariate analysis	
Variables	RR	95% CI	RR	95% CI
Age	0.98	0.97–0.99	0.96	0.92–0.99
Baseline viral load (log_{10})	2.71	1.74–4.25	2.57	1.57–4.22
Dosing (three times a day)	5.58	2.96–10.52	2.64	1.23–5.66
Adherence (< 95%)	2.13	1.22–3.57	1.92	1.10–3.57
Antiretroviral therapy (two NRTI)	3.94	1.96–7.89	2.69	1.17–6.15
Tablets (> 10 a day)	1.85	1.08–3.16	1.11	0.57–2.17
Food restrictions	3.57	1.97–6.49	1.95	0.95–4.03

RR, Risk ratio; CI, confidence interval. NRTI, Nucleoside reverse transcriptase inhibitor.

the cost of monitoring which can be a significant barrier to sustained treatment adherence [19]. Our results suggest that poor patients in sub-Saharan Africa can have successful treatment outcomes when the financial barriers to treatment are removed.

Three times daily therapy was the strongest predictor for both poor adherence and virologic failure in the multivariate analyses. Food restrictions on medication taking and absolute tablet count did not impact on adherence in this cohort and only predicted poor virologic outcomes at a univariate level. Several other studies have also shown that the best improvement in adherence comes with the reduction of dosing frequency from three to two times a day, but have also shown a negative effect of increasing tablet burden on adherence, which this study did not [20,21].

Increasing age was found to correlate with a higher likelihood of remaining on therapy for 48 weeks, as well as improved adherence and virological outcomes. This has been noted in recent literature and is particularly relevant given the rapid increase in HIV seroprevalence in young (19–25-year-old) South Africans [18,22,23].

The home language of each patient was collected as a demographic factor on the hypothesis that patients speaking the same language as the site staff would have an adherence advantage compared to those who were being educated in a second language. People who spoke English, did appear have an adherence benefit. This was unrelated to socio-economic status.

Adherence above 95% to treatment regimens predited virological success. Other factors which impacted on virologic outcome included baseline viral load and regimen type. We noted decreased virologic success with dual nucleoside therapy. Increased viral load at baseline reduced the likelihood of successful viral suppression after 1 year. These factors have been noted to impact similarly elsewhere [18,24].

In this study, clinical stage of HIV at commencement of therapy did not predict adherence, whereas this has been previously reported as a significant predictor [3,20]. Individuals with or without AIDS had the same adherence. Patients in this cohort, in view of their poor socio-economic circumstances and the lack of availability of ART in the South African public sector, may have similar adherence because of the global recognition of the lack of other options for therapy.

In this edition of *AIDS*, Liechty and Bangsberg [25]

argue that the anticipated need to deliver ART as directly observed therapy in developing countries may be premature [3,5,26]. Our results which indicate that poor patients in sub-Saharan Africa can achieve high rates of adherence and viral suppression in the absence of formal adherence intervention support this view.

We used clinic-based pill counts as the primary adherence measure. Whilst there are other measures of adherence that may more accurately reflect pill-taking behavior than clinic-based pill counts [7,8,27–30], this was the only practical and objective method that gave consistent, comparable data across different ART trials. Patients were generally not informed that returns were counted, although this information was not purposefully withheld. Tablet counts are widely used as a marker for adherence [8,11,22,28,29,31]. Pill consumption, however, is not directly measured and therefore the data may represent the upper boundary of actual patient adherence. Patients may not bring in all their medications or some may empty (pill dump) their bottles prior to a clinic visit [22,30]. While these possibilities cannot be excluded, the high rate of viral suppression combined with the significant association between adherence and viral load data suggest the tablet counts in this study correlated with actual adherence. Other investigators have also found a close association between similar clinic-based refill data, as a proxy for adherence, and HIV viral load as well as HIV clinical outcomes, including AIDS and death [12,13, 16,32,33].

The decision to enter patients onto therapy in this study was made on clinical and immunologic criteria; however it is possible that there may have been a selection bias towards patients likely to have better adherence than the general population. ART is not widely available in Cape Town, and, out of large numbers of people with HIV, this cohort was motivated enough to warrant referral from their primary care clinic to a secondary hospital. Subjects on trial also received information and encouragement on a regular basis from medical staff and counselors. This may have enhanced adherence and limit more general applicability.

Most subjects completed 48 weeks of therapy. Subjects who actively withdrew from therapy with in the 48 weeks of the study, tended to do so early in therapy. These patients were generally younger and, although they were not clinically more advanced in terms of disease staging, they tended to have more advanced immunologic and virologic disease. This may have resulted in increased adverse events leading to withdrawal through removal of consent, toxicity, or an HIV-related event. Other demographic factors, such as sex, language and socio-economic status, did not differ between those that withdrew and the rest of the cohort, confirming how difficult it is to predict patient behavior [8,9,34,35].

This study demonstrates that the high levels of adherence required to implement successful ART were achieved in an African cohort without formal adherence intervention. Factors impacting on adherence and virologic outcomes elsewhere were similarly reflected in this cohort. Clinical and virologic benefits were maintained after 1 year. Most importantly, low socioeconomic status was not a barrier to success. Individuals with HIV disease, who could potentially benefit from ART, should not be denied access based on otherwise unsubstantiated expectations of poor adherence.

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Evaluation of nevirapine and/or hydroxyurea with nucleoside reverse transcriptase inhibitors in treatment-naive HIV-1-infected subjects

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Objective: To examine the effect of adding nevirapine (NVP) and/or hydroxyurea (HU) to a triple nucleoside analogue reverse transcriptase inhibitor (NRTI) regimen in terms of efficacy and tolerability.

Methods: HIV-1-infected, treatment-naive adults were randomized, using a factorial design, to add NVP and/or HU to the triple NRTI backbone of zidovudine plus lamivudine plus abacavir. Primary endpoint was treatment failure, defined as having plasma HIV RNA levels > 50 copies/ml after week 24, or discontinuation of randomized treatment. Follow-up was 72 weeks

Results: For the 229 subjects, median plasma HIV-1 RNA was 4.61 $\log_{10}$ copies/ml and median CD4 cell count was 269×10^6 cells/l. NVP users reached plasma HIV-1 RNA < 50 copies/ml more rapidly than subjects using no NVP (log-rank test; $P = 0.011$). In the as-treated analysis, 21.6% of subjects using NVP versus 48.8% using no NVP reached the primary endpoint ($P = 0.013$). In the intent-to-treat analysis, 83.3% of subjects using HU versus 73.0% using no HU experienced treatment failure ($P = 0.060$), while no difference was observed in the as-treated analysis (34.5 versus 36.7%). Differences in the intent-to-treat analysis were accounted for by toxicity: 52.6% of subjects using HU experienced toxicity leading to discontinuation of randomized treatment versus 28.7% of subjects using no HU.

Conclusion: The use of NVP in addition to a triple NRTI regimen improved both short- and long-term antiretroviral efficacy. The use of HU significantly contributed to treatment failure because of toxicity.

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Keywords: HIV, triple therapy, abacavir, nevirapine, hydroxyurea, nucleoside analogue reverse transcriptase inhibitor

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Introduction

Highly active antiretroviral therapy (HAART), consisting of a combination of two nucleoside reverse transcriptase inhibitors (NRTI) and one protease inhibitor, has been shown to reduce the occurrence of opportunistic infections and death significantly, translating into clinical benefit for those patients who have access to therapy [1]. In addition, protease inhibitor-containing HAART has been shown to suppress viral replication in a two-thirds of treated patients for up to 3 years [2]. A combination of two NRTI and the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz has been shown to provide better and more durable suppression of viral replication than a protease inhibitor plus two NRTI in treatment-naive chronically HIV-infected patients through 144 weeks of follow-up [3]. Also, in the Atlantic study [4], the combination of nevirapine (NVP) and two NRTI as first-line therapy for chronic HIV-1 infection displayed similar potent antiviral activity compared with a protease inhibitor-containing or triple NRTI-containing regimen for up to 48 weeks. Guidelines for the initial treatment of chronically HIV-1-infected patients as such have been updated to include the combination of two NRTI plus one NNRTI [5,6].

Recently, the use of the triple NRTI regimen, zidovudine (ZDV) plus lamivudine (3TC) plus abacavir (ABC), in antiretroviral treatment-naive patients has been shown to be at least as effective as the protease inhibitor-containing triple drug regimen ZDV/3TC/indinavir in terms of achieving plasma HIV-1 RNA < 400 copies/ml at 48 weeks [7]. However, when an assay with a lower limit of quantification (LLQ) of 50 copies/ml was used, the protease inhibitor-containing regimen proved superior in terms of suppression of viral replication below this limit in patients with a pre-treatment plasma HIV RNA level $> 100\,000$ copies/ml.

Although the current stated goal of antiretroviral therapy is complete inhibition of viral replication [6], it is becoming increasingly clear this goal cannot be met by standard triple drug regimens. Ongoing residual viral replication was shown to occur, albeit at low levels, in peripheral blood of a group of highly selected patients who had a long-term response to therapy (plasma HIV-1 RNA < 50 copies/ml, conventional assay), when an experimental ultrasensitive assay with a LLQ of 5 copies/ml [8] or 3 copies/ml [9] was used.

Also, on a cellular level, evidence exists of incomplete suppression of viral replication during successful triple drug therapy, such as the continued expression of viral RNA in lymphoid tissue [10], sequence evolution in the viral envelope gene [11], unintegrated circular forms of HIV-1 DNA associated with peripheral blood mononuclear cells [12], and the selection of drug resistance-associated mutations [13].

It was previously shown that implementation of the five-drug triple class regimen ZDV/3TC/ABC/NVP/indinavir led to an accelerated initial decline (over 12 weeks) in plasma HIV RNA level in nine treatment-naive subjects compared with that achieved with a standard triple drug regimen [14]. After 144 weeks of therapy with the same multidrug regimen, improved suppression of viral replication compared with standard therapy was still observed [15].

Around 1998, the use of hydroxyurea (HU) gained popularity, after preliminary studies had shown that the addition of HU to didanosine (ddI) monotherapy resulted in a significant and prolonged suppression of viral replication, both in naive and in ZDV-pretreated patients [16–18]. Randomized, controlled studies followed. In one study [19], the addition of HU to ddI resulted in a significantly higher decrease in plasma HIV-1 RNA compared with ddI monotherapy at 24 weeks, while in another [20], HU was shown to improve the antiviral activity of the dual NRTI combination ddI plus stavudine (d4T) over a 12 week period.

The use of HU has also been studied in the context of triple combination drug therapy [21,22]. In the 3D study [21], 147 patients were randomized 2 : 1 to add HU (600 mg twice daily) or placebo to efavirenz plus ddI plus d4T. At 48 weeks, there was no difference in efficacy among treatment-naive patients. In addition, 31 patients who added HU experienced significant adverse events compared with 19 in the placebo group. In the ACTG 5025 study [22], subjects who had been successfully treated with ZDV/3TC/indinavir for at least 6 months were randomized to continue this treatment or to switch to d4T/ddI/indinavir with or without HU 600 mg twice daily. No virological or immunological differences were found, but there was a higher incidence of toxicities with HU, including two deaths caused by pancreatitis. In both studies [21,22], the use of HU was discontinued prematurely by the Drug Safety and Monitoring Board because of increased toxicity. When the CHARM study was designed, data on the efficacy and toxicity of HU when used as a component of triple antiretroviral combination drug therapy was scarce.

It is known that the main side effect of HU is bone marrow toxicity. However, this toxicity is dose dependent. At the low doses commonly used for HIV infection (1–1.2 g daily), bone marrow toxicity (anaemia and neutropenia) occurs in 5–7% of patients [19,20,23]. Most is a grade I–II toxicity, not requiring dosage adjustment or discontinuation of treatment. Grade III–IV toxicity is rare. Other toxicities that have

been described include alopecia, gastrointestinal disorders (nausea and vomiting) and transient elevation of liver enzymes [24].

The observation that HU enhances the *in vitro* anti-HIV activity of a thymidine analogue (d4T) and cytidine analogue (3TC) NRTI by increasing their intracellular phosphorylation [25,26] provides a rationale for combining HU with these drugs. However, there is no *in vivo* proof that this is the mechanism. The clinical benefit of adjunctive HU therapy in combination with other nucleosides remains an important research question. In addition, given the unique features of HU, such as ease of administration, limited drug interactions and low cost, its possible role in simplified treatment regimens that target populations in developing countries is particularly appealing.

The aim of the study was to investigate the effect of adding NVP and/or HU to the triple NRTI regimen ZDV/3TC/ABC in terms of efficacy and tolerability. In addition, the study examined the effect of adjuvant prednisolone during the first 2 weeks of therapy on the development of hypersensitivity reactions associated with ABC and NVP. Results of this additional analysis have been published elsewhere [27].

Methods

Study participants

The main eligibility criteria for inclusion in this study were documented HIV-1 infection by a licensed HIV-1 antibody enzyme-linked immunosorbent assay (ELISA), no previous exposure to antiretroviral drug therapy (prior NRTI exposure for less than 2 weeks was permitted), plasma HIV-1 RNA at least 5000 copies/ml within 4–12 weeks of study drug administration and a negative serum pregnancy test (human beta-chorionic gonadotrophin) at screening for women of child-bearing potential. There was no entry criterion for CD4 cell count. Patients were excluded they were known to have experienced clinically relevant pancreatitis or neuropathy during the 6 month period preceding screening; if they had hepatic dysfunction evident by grade III or IV hyperbilirubinaemia (ACTG toxicity grading scale) or aspartate transaminase > 5 times the upper limit of normal (local laboratory limits); if they had renal failure requiring dialysis; or if any of the following laboratory parameters were present during the 4 week period prior to study drug administration: a haemoglobin concentration < 10.0 g/dl (6.3 mmol/l) for men and < 9.0 g/dl (5.7 mmol/l) for women, neutrophil count $< 1 \times 10^9$ cells/l, thrombocyte count $< 75 \times 10^9$ cells/l and a serum pancreatic amylase > 1.5 times the upper limit of normal. Subjects were also excluded if they were pregnant or breastfeeding; had received radiation therapy or cytotoxic chemotherapeutic agents within 30 days of study drug administration (or had an anticipated need for such treatment), with the exception of localized treatment for Kaposi's sarcoma; had received treatment with immunomodulating agents such as systemic corticosteroids, interleukins or interferons within 30 days of study drug administration; abused alcohol; or had a severe HIV-related or non-HIV-related disease incompatible with study treatment, as judged by the investigator.

Study design

This 72-week open-label, randomized, multicentre study was conducted at 21 sites in South Africa, Canada, western and eastern Europe. The institutional review boards and independent ethics committee at each site approved the study and all patients gave written informed consent before initiating the study. The study had a factorial design. All patients who entered the study received ZDV, 3TC and ABC. When possible, ZDV and 3TC were given as the combination tablet Combivir®. In this factorial design, subjects were first allocated to the addition or not of NVP. Second, subjects were randomized to the addition or not of HU. Finally, there was a third randomization to the addition or not of prednisolone during the first 2 weeks of the study, with the intention to prevent hypersensitivity reactions associated with NVP and ABC. Study dosages were ZDV 300 mg twice daily, 3TC 150 mg twice daily, ABC 300 mg twice daily, HU 500 mg twice daily, NVP 200 mg once daily during the first 14 days of treatment, followed by 200 mg twice daily from day 15 onwards, and prednisolone 40 mg once daily during the first 14 days only. Subjects were to continue their randomized treatment until 72 weeks follow-up unless they met protocol-defined criteria for premature treatment discontinuation, such as treatment failure, pregnancy, withdrawal of written consent or experienced treatment-related adverse events sufficiently severe to discontinue randomized treatment prematurely.

Study monitoring

Study visits were scheduled for week −4 (screening visit), week −2, week 0 (baseline visit and start of study medication), and weeks 2 (dose escalation of NVP and discontinuation of prednisolone), 4, 6, 12, 24, 36, 48, 60 and 72. At week −4, subjects signed informed consent and were screened to see whether they met all entry criteria. If not previously recorded, seropositive status for HIV-1 infection was confirmed by a licensed HIV-1 antibody ELISA test. A serum pregnancy test was performed where applicable and was repeated at weeks 12, 24, 36 and 48. Current medical conditions, HIV-associated conditions, classification according to the Center for Disease Control (CDC), weight, the use of concurrent medications and/or blood products were recorded, and haematology, clinical chemistry, lympho-

cyte subsets (using flow cytometry), quantitative HIV-1 RNA measurement [Roche Amplicor version 1.5 (Roche Molecular Systems, Branchburg, New Jersey, USA) with a LLQ of 50 copies/ml] were measured at each study visit. A plasma sample was also collected. All laboratory assays were performed at the local laboratory with the exception of HIV-1 RNA and sample storage, which were carried out at a central laboratory. At week −2, demography, risk factors or mode of transmission and height were recorded and serology for hepatitis B and C was done. Safety assessments were based on evaluations of medical history, vital signs, haematology, clinical chemistry, urinalysis and clinical adverse experiences. The last were evaluated using the ACTG toxicity grading tables for grading severity of adult adverse experiences. Clinical Research Associates monitored the different study sites for consistency of data and laboratory tests performed at the local laboratories. An independent Data and Safety Monitoring Board reviewed safety and efficacy data once all subjects completed 24 weeks follow-up.

Outcome measurements

A factorial study design was chosen to allow for independent consideration of the effect of adding HU and NVP on treatment outcome. To test whether this assumption was valid, an interaction factor (NVP × HU) was calculated to examine the appropriateness of pooling data for analyses. Comparisons in both the efficacy and safety analyses were based on study groups, categorized as follows: NVP use/non-use and HU use/non-use, as opposed to the different treatment arms in the study.

The primary efficacy analysis was a comparison of the different study groups with respect to the proportion of subjects with treatment failure at week 72. The population considered for the primary analysis was the intention-to-treat population and included all randomized subjects, categorizing those subjects who withdrew from the study for any reason as treatment failures. Treatment failure was defined as having a plasma HIV RNA level $\geqslant 50$ copies/ml after week 24 or discontinuation of randomized treatment. Missing plasma HIV-1 RNA measurements were considered to be > 50 copies/ml, unless the measurement preceding and following the missing measurement were both < 50 copies/ml.

In the secondary efficacy analysis, a comparison was made between the different study groups with respect to the proportion of subjects with treatment failure in the as-treated population, consisting only of those subjects still on randomized treatment at week 72. Additionally, the $\log_{10}$ copies/ml reduction in plasma HIV RNA from baseline was calculated by using an uncensored method, where plasma HIV RNA levels $\leqslant 50$ copies/ml (LLQ of the assay) were set at 50 copies/ml. Also included in the secondary efficacy analyses were the time to reach a plasma HIV RNA level $\leqslant 50$ copies/ml and the time to first virological failure, both in the intention-to-treat population. For the latter analysis, only subjects who had reached a plasma HIV RNA level $\leqslant 50$ copies/ml on or before week 24 were included. Logistic regression analysis was performed in the intention-to-treat population to determine the influence of NVP use, HU use, gender, age, baseline CD4 cell count and baseline plasma HIV RNA level on treatment outcome at week 72.

The change in CD4 cell count from baseline was calculated by study group in the intention-to-treat population. For the purpose of the drug safety analysis, all serious adverse events and additional grade III (severe) and grade IV (life-threatening) adverse events considered to be possibly, probably or almost certainly attributable to the use of the study medication were tabulated by study group. In addition, all grades of treatment-emergent laboratory toxicity, as measured by the ACTG toxicity scale, were summarized by study group. When an event occurred more than once in the same subject, only the highest toxicity grade measured was included in the analysis. A summary was made, by study group, of all adverse events that led to a discontinuation or change in randomized treatment.

Statistical analysis

All analyses were carried out according to a predefined data analysis plan using SAS statistical package version 8.2 (SAS Institute, Cary, North Carolina, USA). The study had 80% power to detect a difference in the proportions of subjects with treatment failure of 0.15 to 0.20 with a significance level of 0.05 between the treatment groups. Differences in proportions were analysed by the chi-squared test. In the logistic regression analysis, variables that were statistically significant in the univariate analysis were used in the multivariate analysis. To test the factorial design assumption of independence of the treatment groups, the interaction between NVP and HU use was tested in a multivariate model, which included NVP use, HU use and their interaction. For analysis of the time to reach plasma HIV RNA < 50 copies/ml and to first virological failure, Kaplan–Meier estimates were produced and tested by a log rank test. The change in CD4 cell count from baseline was analysed by using a 'proc mixed' procedure, which accommodates repeated measurements and estimates mean values by a least square analysis. Treatment group and time were used as covariates in an unstructured matrix.

Results

Baseline characteristics

A total of 229 subjects were enrolled in the study between August 1999 and June 2000. Baseline char-

acteristics are shown in Table 1 and study design in Fig. 1. The two different study groups were balanced with respect to demographic and baseline characteristics.

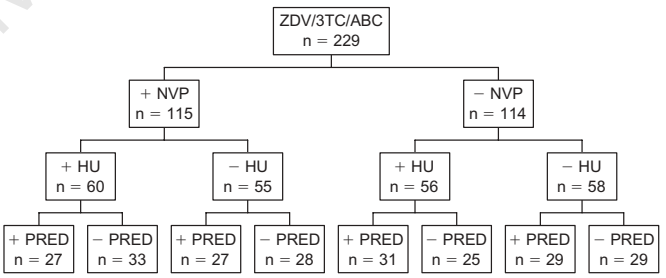
Subject disposition at week 72

Seven subjects never received randomized, assigned treatment. Thirty-eight subjects prematurely discontinued the study after having received assigned treatment (Fig. 1). Reasons included patient request (n = 18), lost to follow-up (n = 18) and other reasons (n = 2). A total of eight deaths occurred during the study. Four of these deaths were AIDS related (one each of cryptococcal meningitis, tuberculous meningitis, extensive

Table 1. Summary of demographic and baseline characteristics by study group.

	NVP arm		Hu arm	
Characteristics	Without NVP	NVP	Without HU	HU
No.	114	115	115	114
Mean age [years (SD)]	34.4 (10.1)	35.3 (9.3)	35.1 (9.7)	34.5 (9.7)
Sex (% male)	67	75	67	72
Mean weight [kg (SD)]	67.6 (12.0)	67.8 (12.9)	67.9 (13.2)	67.5 (11.8)
Median CD4 cell count [$\times 10^6$ cell/l (IQR)]	254 (121–400)	273 (146–383)	256 (146–383)	272 (92–400)
Median HIV-1 RNA [$\log_{10}$ copies/ml (IQR)]	4.73 (4.38–5.38)	4.56 (4.33–5.21)	4.63 (4.31–5.23)	4.61 (4.36–5.37)
Mean BMI [kg/m^2 (SD)]	23.0 (3.5)	23.3 (3.6)	23.4 (3.7)	22.8 (3.4)
CDC class (%)				
A	49	55	49	54
B	32	27	29	30
C	19	18	22	16
Risk group (%)				
Homosexual/bisexual	31	34	30	34
Heterosexual	51	45	50	46
Intravenous drug use	14	17	16	15
Blood	–	–	–	–
Other	1	0	1	0
Unknown	3	4	3	5
Region (%)[a]				
Western Europe	35	40	38	37
Eastern Europe	27	24	24	27
South Africa	38	36	38	36

NVP, nevirapine; HU, hydroxyurea; BMI, body mass index; IQR, interquartile range; CDC, Centers for Disease Control and Prevention.[a]One patient was included in Canada.



Discontinued before start:	–	1	1	1(†)	–	1	2	1
Discontinued after start								
• patient request	4	2	2	3	3	2	1	1
• lost to follow-up	–	2	2	3	3	2	3	3
• death	–	1	–	1	1	1	3	–
• other	–	1	–	–	–	–	–	1
On study at week 72:	23	26	24	18	22	19	21	24
On treatment at week 72:	7	7	12	11	10	5	13	13

Fig. 1. Patient disposition at week 72. ZDV, zidovudine; 3TC, lamivudine; ABC, abacavir; NVP, nevirapine; HU, hydroxyurea; PRED, prednisolone.

Kaposi sarcoma and wasting syndrome). The other deaths were unrelated to HIV disease: one subject died of metastatic carcinoma (aetiology unknown), one committed suicide, one died of mesentery infarction and one of aplastic anaemia. It is possible that the aplastic anaemia was related to ZDV and HU use. After 72 weeks, 177 patients were still in the study, of whom 78 (44%) were still on randomized treatment (Fig. 1).

Efficacy analysis

No significant interaction was found between NVP use and HU use on treatment outcome, odds ratio 1.36 [95% confidence interval (CI), 0.37–4.99], validating the assumption of independence of effects and allowing for pooling of data (Table 2).

Primary efficacy endpoint

The population considered was the intention-to-treat population (n = 229). Treatment failure in the NVP group was 74.8% (95% CI, 65.8–82.4) in those taking NVP versus 81.6% (95% CI, 73.2–88.2) for those not taking NVP ($P = 0.213$); in the HU group, it was 83.3% (95% CI, 75.2–89.7) in those taking HU versus 73.0% (95% CI, 64.0–80.5) in those not taking HU ($P = 0.060$) (Table 3).

Secondary efficacy endpoints

In the as-treated population (n = 78), in the NVP group, 21.6% (95% CI, 9.8–38.2) of subjects adding NVP versus 48.8% (95% CI, 32.9–64.9) of subjects not adding NVP experienced treatment failure ($P = 0.013$), while in the HU group there was no significant difference in treatment failure rate between subjects ($P = 0.841$).

Overall, subjects experienced a median reduction of 2.78 $\log_{10}$ copies/ml HIV-1 RNA from baseline at week 72, while no significant differences were seen between subjects in the different treatment groups. In the NVP group, the proportion of subjects reaching plasma HIV-1 RNA < 50 copies/ml increased more rapidly over time in NVP users than in non-users (log rank test; $P = 0.011$; Fig. 2, while in the HU group no significant difference was observed between subjects.

Kaplan–Meier estimates showed no significant differences between the groups with respect to the time to first virological failure (NVP use versus non-use: log-rank $P = 0.238$; HU use versus non-use: log-rank $P = 0.493$). In the univariate logistic regression analysis, none of the parameters tested significantly increased the odds ratio of experiencing treatment failure (Table 2). However, a trend was observed for HU use: odds ratio 1.85 (95% CI, 0.97–3.51).

The change from baseline in absolute CD4 cell count in time by study group is shown in Fig. 3. There were no treatment effects observed in the NVP group. In the HU group, subjects taking no HU experienced a significantly higher increase in absolute CD4 cell count than did non-users ($P < 0.001$). However, no significant differences in CD4 cell percentage increase over time was seen between subjects in either treatment

Table 2. Univariate analysis for treatment failure.

	OR	Interaction
NVP	0.67 (0.36–1.26)	0.57 (0.25–1.31)
HU	1.85 (0.97–3.51)	1.59 (0.60–4.20)
Interaction NVP × HU		1.36 (0.37–4.99)
Sex (female)	0.98 (0.50–1.95)	
Age (years)	1.02 (0.98–1.05)	
Baseline CD4 ($\times 10^6$ cell/l)	1.03 (0.88–1.20)	
Baseline HIV-1 RNA ($\log_{10}$ copies/ml)	1.15 (0.72–1.81)	

OR, odds ratio; CI, confidence interval; NVP, nevirapine; HU, hydroxyurea.

Table 3. Percentage of patients with treatment failure at week 72.

	Intention-to-treat population			As-treated population		
Study group	No.	Failure [% (95% CI)	*P* value	No.	Failure [% (95% CI)	*P* value
NVP arm						
Without NVP	114	81.6 (73.2–88.2)		41	48.8 (32.9–64.9)	
NVP	115	74.8 (65.8–82.4)	0.213	37	21.6 (9.8–38.2)	0.013
HU arm						
Without HU	115	73.0 (64.0–80.9)		49	36.7 (23.4–51.7)	
HU	114	83.3 (75.2–89.7)	0.060	29	34.5 (17.9–54.3)	0.841

CI, confidence interval; NVP, nevirapine; HU, hydroxyurea.

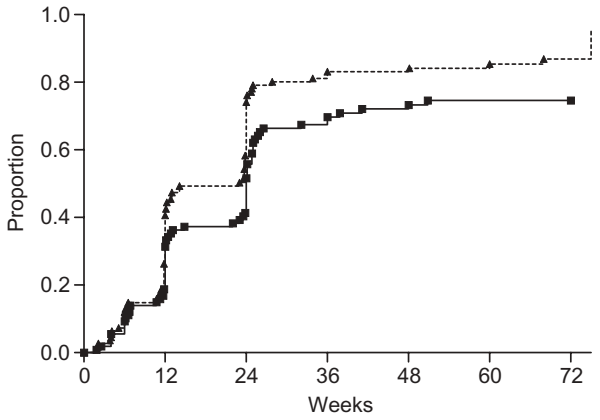


Fig. 2. Kaplan–Meier curve showing the proportion of subjects reaching plasma HIV-1 RNA < 50 copies/ml over time for the nevirapine arm. —, patients not using nevirapine; - - -, patients using nevirapine. Log-rank $P = 0.011$.

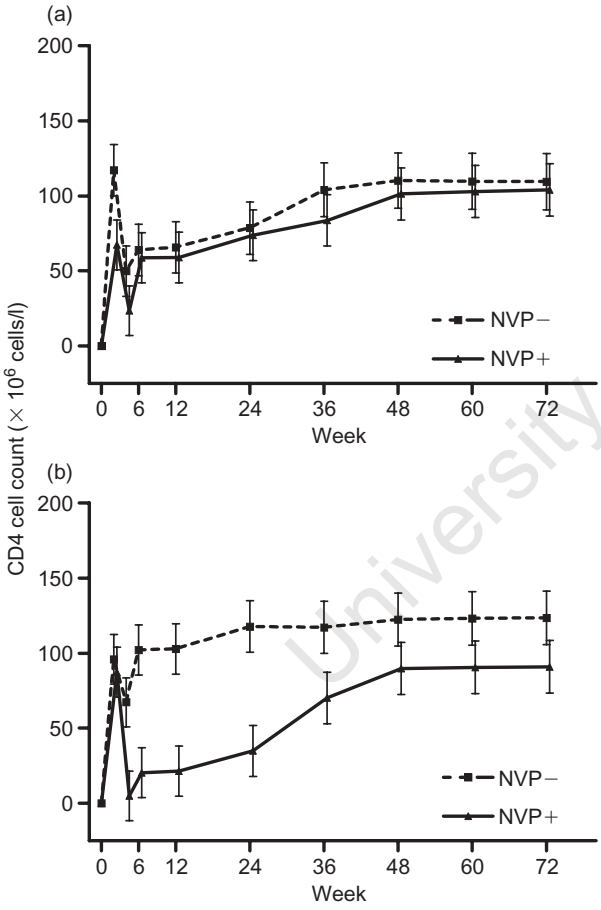


Fig. 3. Change from baseline in CD4 cell count over time summarized by study group. NVP−, patients not using nevirapine; NVP+, patients using nevirapine; HU−, patients not using hydroxyurea; HU+, patients using hydroxyurea.

group. Disease progression, defined as deterioration in CDC class or death, occurred in 14% of subjects (intention-to-treat). No differences were observed between the different treatment groups.

Safety analysis

One or more serious adverse event, or additional grade III and IV adverse event that was considered treatment emergent, was observed in 59 subjects (51.3%) taking NVP versus 37 subjects (32.5%) not taking NVP in the NVP arm ($P = 0.003$), and 54 subjects (47.4%) taking HU versus 42 subjects (36.5%) not taking HU in the HU arm ($P = 0.096$) (Table 4). In the NVP arm, the following events were reported more frequently in NVP users than in non-users: rash (5.2% versus 1.8%), liver toxicities (11.3% versus 1.8%), hypersensitivity reaction to ABC and/or NVP (16.5% versus 5.3%) and gastrointestinal events (13.9% versus 7.9%). In the HU arm, gastrointestinal events (14.9% versus 7.0%), anaemia (16.7% versus 2.6%), neutropenia (17.5% versus 9.6%) and pancytopenia (1.8% versus 0%) were reported more frequently in users than in non-users. One or more adverse events that led to discontinuation of randomized treatment occurred in 60 subjects (52.6%) adding HU versus 33 subjects (28.7%) not adding HU in the HU arm ($P < 0.001$), and in 55 subjects (47.8%) adding NVP versus 38 subjects (33.3%) not adding NVP in the NVP arm ($P = 0.026$). In September 2001, the Drug Safety and Monitoring Board unanimously recommended that HU be discontinued because of the unacceptably high proportion of subjects experiencing adverse events that led to a change or discontinuation of randomized treatment. The proportion of subjects with laboratory abnormalities (all grades) was similar between the different treatment groups (data not shown), with the exception of gamma-glutamyl transaminase toxicity, which occurred significantly more frequent in NVP users ($P = 0.04$).

Discussion

This randomized trial is the first that has allowed independent consideration of the effect of adding NVP and/or HU to a triple NRTI drug regimen in terms of antiviral efficacy and tolerability; this was achieved by the factorial design.

In the NVP group, subjects who added NVP experienced an accelerated decay in plasma HIV-1 RNA. In Fig. 3, it can be seen that the proportion of subjects reaching 'undetectability' of HIV-1 RNA over time increased more rapidly in NVP users, suggesting that the addition of NVP increased the potency of the triple NRTI regimen. The as-treated analysis, which is a reflection of virological efficacy, confirms this observation. The addition of NVP failed to improve treatment outcome in the intention-to-treat population. This was because a significant proportion of subjects who added NVP experienced one or more adverse events that necessitated a change in randomized treatment. By

Table 4. All serious and additional grade III and IV adverse events, summarized by study group.

	No. in NVP arm				No. in HU arm			
	Without NVP		NVP		Without HU		HU	
Adverse event	AE 3/4	SAE	AE 3/4	SAE	AE 3/4	SAE	AE 3/4	SAE
Rash	0	2	3	3	1	5	2	0
Hypersensitivity reaction[a]	0	6	0	19	0	14	0	11
Gastrointestinal events	1	8	10	6	3	5	8	9
Pancytopenia	1	0	0	1	0	0	1	1
Anaemia	1	17	1	2	1	1	1	18
Neutropenia	11	1	18	1	11	0	18	2
Constitutional events	2	4	6	4	6	5	2	3
Urogenital events	0	3	1	1	0	4	1	0
Liver toxicities	2	0	13	0	8	0	7	0
Neurological events	2	6	3	7	4	7	1	6
Cryptococcal disease	0	1	0	1	0	2	0	0
Other skin event	0	2	0	2	0	2	0	2
ENT disease	0	2	0	0	0	0	0	2
Respiratory events	0	2	0	2	0	3	0	1
Sepsis/bacteraemia	0	0	0	2	0	1	0	1
Other	0	2	0	4	0	2	0	4
Patients with one or more events	37		59		42		54	
P value[b]	0.003				0.096			

NVP, nevirapine; HU, hydroxyurea, AE 3 /4, grade III and IV adverse event; SAE, serious adverse event; ENT, ear nose throat.
[a]To abacavir and/or NVP.
[b]*P* value for comparison of with and without drug in trial arm.

definition, these subjects were considered treatment failures.

Hypersensitivity reactions to ABC and/or NVP were reported more frequently in subjects allocated to the use of NVP (n = 19; non-users reported 7). As clinical symptoms of NVP- and ABC-associated hypersensitivity reactions overlap, a clear distinction between the two cannot reliably be made. Furthermore, none of the study arms consisted of the use of NVP without ABC, making adequate comparisons between NVP- and ABC-associated hypersensitivity reactions impossible. Therefore, these events were treated as a composite endpoint. In addition, the outcome of ABC hypersensitivity reactions can be fatal if the reaction is not recognized and treatment with ABC discontinued. It is possible that the open-label design of the study may have introduced a bias in reporting the occurrence of hypersensitivity reactions.

More subjects allocated to the use of HU experienced treatment failure at week 72 (intention-to-treat population), although the difference between subjects did not reach significance. The observed difference between subjects resulted from the more frequent occurrence of adverse events in subjects using HU. In the as-treated population, treatment failure was the same in those that did and did not use HU, suggesting that HU use did not improve the antiviral efficacy of the triple NRTI regimen.

The main toxicity encountered in clinical practice with HU is bone marrow suppression, which can lead to neutropenia, anaemia and/or thrombocytopenia [24]. In the HU group, anaemia and neutropenia of severe or life-threatening intensity were reported in 20 and 21 subjects who were allocated to HU versus 3 and 12 subjects not taking the drug, respectively. The myelosuppressive effect of HU has been shown to be enhanced if HU is used in association with other haematotoxic drugs such as ZDV or cotrimoxazole [17]. We are able to confirm this observation.

In our study, 85 (74.6%) of the 114 subjects originally allocated to the use of HU discontinued the drug by week 72. These numbers are comparable with those reported in the long-term follow-up of subjects in the ddH study [28], where 75% of the 72 patients originally randomized to HU in combination with d4T/ddI and 80% of the 30 patients who elected to add HU after week 12 discontinued the drug 24 months after the start of the trial. In the as-treated population, we have observed a treatment failure rate in the order of 35% in subjects taking HU. Once again, these results are very similar to those reported in the ddH study [28], where 9/18 (50%) of subjects still taking ddI/d4T/HU and 5/15 (33%) taking ddI/d4T had plasma HIV-1 RNA levels > 200 copies/ml after 72 weeks of follow-up.

HU enhances the antiviral activity of adenosine analogues, mainly that of ddI, by decreasing the intracellular dATP pool, which is the natural competitor of ddI at the DNA elongation process [17,25]. The benefits obtainable with the association of HU with other

NRTI are smaller as the reduction of paired dNTP levels is less effective than for dATP competitive inhibitors [29,30]. This is one possible explanation why addition of HU to the triple NRTI regimen ZDV/3TC/ABC did not improve the antiviral efficacy of the regimen.

In a systematic overview of results from clinical trials involving triple drug therapy [31], the overall estimated percentages of patients with plasma HIV-1 RNA < 50 copies/ml at 48 weeks by drug class were 46% for a regimen containing a protease inhibitor, 51% for one containing a NNRTI, and 45% for triple NRTI. The overall failure rates in our study were much higher, especially for a treatment-naive population. The primary endpoint of the study was a composite of failure of virological suppression and discontinuation of randomized treatment for any reason. This explains the high failure rate in the intention-to-treat population. In addition, approximately 35% of subjects in the NVP and HU groups failed in the as-treated population. It is possible that this was caused by adherence problems. Although adherence was measured in the study, insufficient data were collected for analysis.

From an immunological viewpoint, a significant increase in the CD4 cell count in subjects was observed over time in both treatment groups. Subjects who used HU experienced a slower rate of increase in absolute CD4 cell numbers, with no difference in increase in CD4 cell percentage compared with non-users. This observation is similar to reports from a previous study in which HU was shown to decrease lymphocyte counts by means of its cytostatic effect [20].

The present study has some limitations. It had an open-label design so a reporting bias for adverse events could have been introduced. In addition, we have only limited data on adherence. Therefore, the question of whether the high failure rate observed in the as-treated population was the result of poor adherence to therapy remains unanswered.

In conclusion, our findings suggest that the addition of NVP to the triple NRTI regimen ZDV/3TC/ABC resulted in an accelerated decay in plasma viraemia and enhancement of the antiviral efficacy of the regimen. However, the higher incidence of hypersensitivity reactions in subjects who added NVP is an argument for the sequential introduction of ABC and NVP when these two agents are used in combination. The addition of HU significantly contributed to treatment failure and was poorly tolerated because of toxicity. It is regrettable that HU has proven to be that toxic, as the low cost of the drug makes it an ideal candidate for use in resource-poor settings, where effective antiretroviral therapy remains beyond the reach of most HIV-infected persons because of its cost.

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Articles

Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study

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Summary

Background The 2NN Study was a randomised comparison of the non-nucleoside reverse-transcriptase inhibitors (NNRTI) nevirapine and efavirenz.

Methods In this multicentre, open-label, randomised trial, 1216 antiretroviral-therapy-naive patients were assigned nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, or nevirapine (400 mg) and efavirenz (800 mg) once daily, plus stavudine and lamivudine, for 48 weeks. The primary endpoint was the proportion of patients with treatment failure (less than 1 $\log_{10}$ decline in plasma HIV-1 RNA in the first 12 weeks or two consecutive measurements of more than 50 copies per mL from week 24 onwards, disease progression [new Centers for Disease Control and Prevention grade C event or death], or change of allocated treatment). Analyses were by intention to treat.

Findings Treatment failure occurred in 96 (43·6%) of 220 patients assigned nevirapine once daily, 169 (43·7%) of 387 assigned nevirapine twice daily, 151 (37·8%) of 400 assigned efavirenz, and 111 (53·1%) of 209 assigned nevirapine plus efavirenz. The difference between nevirapine twice daily and efavirenz was 5·9% (95% CI –0·9 to 12·8). There were no significant differences among the study groups in the proportions with plasma HIV-1 RNA concentrations below 50 copies per mL at week 48 (p=0·193) or the increases in CD4-positive cells (p=0·800). Nevirapine plus efavirenz was associated with the highest frequency of clinical adverse events, and nevirapine once daily with significantly more hepatobiliary laboratory toxicities than efavirenz. Of 25 observed deaths, two were attributed to nevirapine.

Interpretation Antiretroviral therapy with nevirapine or efavirenz showed similar efficacy, so triple-drug regimens with either NNRTI are valid for first-line treatment. There are, however, differences in safety profiles. Combination of nevirapine and efavirenz did not improve efficacy but caused more adverse events.

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Introduction

Several randomised clinical trials in antiretroviral-therapy-naive patients have shown that a regimen based on a non-nucleoside reverse-transcriptase inhibitor (NNRTI) is at least as effective as a regimen that includes a protease inhibitor.[1–3] During the past few years, the use of NNRTI-based antiretroviral therapy as the first regimen of choice has become increasingly popular. Among the reasons for this change are the lower pill burden of such a regimen and the perceived toxicity associated with protease-inhibitor-based regimens. Furthermore, NNRTI-based antiretroviral therapy does not necessitate any restrictions on food intake. These factors can contribute to better adherence to therapy by the patient, which is crucial for a sustained effect of treatment.[4–6]

The two most used NNRTI drugs are nevirapine and efavirenz. No large randomised comparison between these two drugs has yet been undertaken. Several large cohort-studies have suggested that efavirenz is more effective than nevirapine.[7–9] However, interpretation of cohort studies might be hampered by the possible biases that are inherent in this design.

We report the results of the 2NN Study, a large randomised trial in patients with chronic HIV-1 infection who had not previously received antiretroviral therapy, comparing the efficacy and safety of treatment with two nucleoside-analogue reverse-transcriptase inhibitors (NRTI) and either nevirapine once daily, nevirapine twice daily, efavirenz, or the combination of nevirapine and efavirenz. The study addressed several questions. First, it compared the standard dose of nevirapine (twice daily) with efavirenz. Second, it compared the standard twice-daily regimen of nevirapine with a once-daily dose. The

use of once-daily dosing helps to simplify treatment. Although once-daily dosing is used widely, no large formal comparison with nevirapine twice daily has been done. Third, the study assessed the relative merit of combining the two NNRTI drugs. This combination has never been investigated in a clinical setting, and in-vitro data on the combination are inconclusive.[10,11]

Methods

Participants

Study participants were recruited during regular visits to HIV treatment centres in North and South America, Australia, Europe, South Africa, and Thailand. Eligible patients were antiretroviral-therapy naive, chronically infected with HIV-1, and aged at least 16 years, and had a plasma concentration of HIV-1 RNA of more than 5000 copies per mL. There was no inclusion criterion for the number of CD4-positive T lymphocytes or disease stage. Reasons for exclusion were: pregnancy or lactation; haemoglobin concentration less than 6·3 mmol/L in men or 5·7 mmol/L in women; neutrophil count less than 1×10^9/L; platelet count less than 75×10^9/L; serum amylase more than 2·0 times the upper limit of normal in combination with serum lipase more than 1·5 times the upper limit of normal; aspartate aminotransferase more than 5·0 times the upper limit of normal; or bilirubin more than 2·5 times the upper limit of normal; history of clinical pancreatitis or neuropathy within the previous 6 months; renal failure necessitating dialysis; radiotherapy, cytotoxic, or immunomodulating treatment within the month preceding the start of study or the expected need for it; infection with HIV-2; or likely non-adherence as judged by the investigator.

The study was approved by the ethics review bodies in the participating countries, and all patients gave written informed consent.

Design and interventions

All patients received stavudine 40 mg twice daily (30 mg twice daily if weight was less than 60 kg) and lamivudine 150 mg twice daily. They also received nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily, or a combination of nevirapine 400 mg once daily and efavirenz 800 mg once daily. The higher dose of efavirenz when it is used in combination is recommended because efavirenz concentrations are lowered when the drug is used in normal dosing in the presence of nevirapine.[12] Nevirapine was given as 200 mg once daily for the first 2 weeks.

At the central study coordination centre (International Antiviral Therapy Evaluation Center, Amsterdam), a treatment allocation sequence was generated by use of the minimisation variables CD4-positive T-cell count (≤350 *vs* >350 cells per μL) and study region. Treatment allocation was stratified by baseline plasma HIV-1 RNA concentration (≤30 000 copies per mL *vs* >30 000 copies per mL).

The study started with three comparison groups, an estimated sample size of 450, and a treatment allocation ratio of 1:1:1 for nevirapine once daily, efavirenz, and nevirapine plus efavirenz. The group assigned nevirapine twice daily was added 5 months later because another study had found that the efficacy of nevirapine was related to the minimum concentration[13] and raised the issue of whether once-daily nevirapine resulted in a high enough minimum concentration.

The focus of the trial shifted to the comparisons of nevirapine twice daily versus efavirenz (testing for equivalence) and of nevirapine once daily versus twice daily (testing for superiority). For these comparisons, the treatment allocation probabilities were recalculated to arrive at the end of the inclusion period at a 1:2:2:1 allocation ratio for nevirapine once daily, nevirapine twice daily, efavirenz, and nevirapine plus efavirenz, and an estimated sample size of 1200 patients. The decision to add the fourth treatment group was taken without knowledge of outcomes for patients already enrolled. For 828 (68%) of the total 1216 patients, treatment allocation could have been to one of four study groups. Treatment allocation was done at the central study coordination centre, concealed from the investigator before enrolment, and implemented by sending a fax to the study site. Generation and implementation of the allocation sequence were fully separated. There was no masking after treatment allocation.

By protocol, patients were not allowed to change any component of their allocated treatment for more than 5% of the follow-up time. The only exception was a change of stavudine or lamivudine for reason of toxicity, provided that two NRTI drugs were used at all times. Women who became pregnant while taking efavirenz were switched to nevirapine and could remain in the study, but they were judged to have had a change of allocated treatment. The use of prophylactic medication for opportunistic infections was at the discretion of the treating physician.

Follow-up and assessments

Participants were assessed at study weeks –6 (screening), 0 (start of treatment), 2, 4, 8, 12, 24, 36, and 48. At these visits, we measured plasma HIV-1 RNA concentration, counted CD4-positive T cells, and recorded clinical and laboratory adverse events. Further monitoring of plasma HIV-1 RNA concentration was done at day 3, week 1, and week 18. Plasma samples for measurement of HIV-1 RNA concentration were frozen at –70°C and transported to a central laboratory (LabCorp, Research Triangle Park, NC, USA), where the RNA concentration was measured with an Ultrasensitive Roche Amplicor 1.5 (Roche Diagnostics, NJ, USA) with a lower limit of quantification of 50 copies per mL. All other laboratory tests were done at local or regional laboratories by standard techniques or enzymatic assays. The Virtual Central Laboratory (VCL, Zeist, Netherlands) selected these laboratories, assured the quality, and normalised all results. The toxicity grading scale of the AIDS Clinical Trial Group was used for the reporting of clinical and laboratory adverse events.[14]

Outcome measures

The primary efficacy outcome was the proportion of patients with treatment failure, defined as a composite endpoint with three components: virology (a decline of less than 1 $\log_{10}$ in plasma HIV-1 RNA concentration within the first 12 weeks or two consecutive measurements ≥50 copies per mL from week 24 onwards; a single plasma HIV-1 RNA concentration ≥50 copies per mL at week 48 constituted a failure); disease progression (Centers for Disease Control and Prevention grade C event from 8 weeks onwards diagnosed according to published guidelines,[15] or death); and therapy change (non-allowable change of allocated treatment). Secondary endpoints were the proportion of patients with virological failure (never having a plasma HIV-1 RNA concentration <50 copies per mL, or two consecutive measurements ≥50 copies per mL after having had a concentration below the cut-off); the proportion of patients with plasma HIV-1 RNA concentrations below 50 copies/mL at each study week; the change in CD4-positive cells between the

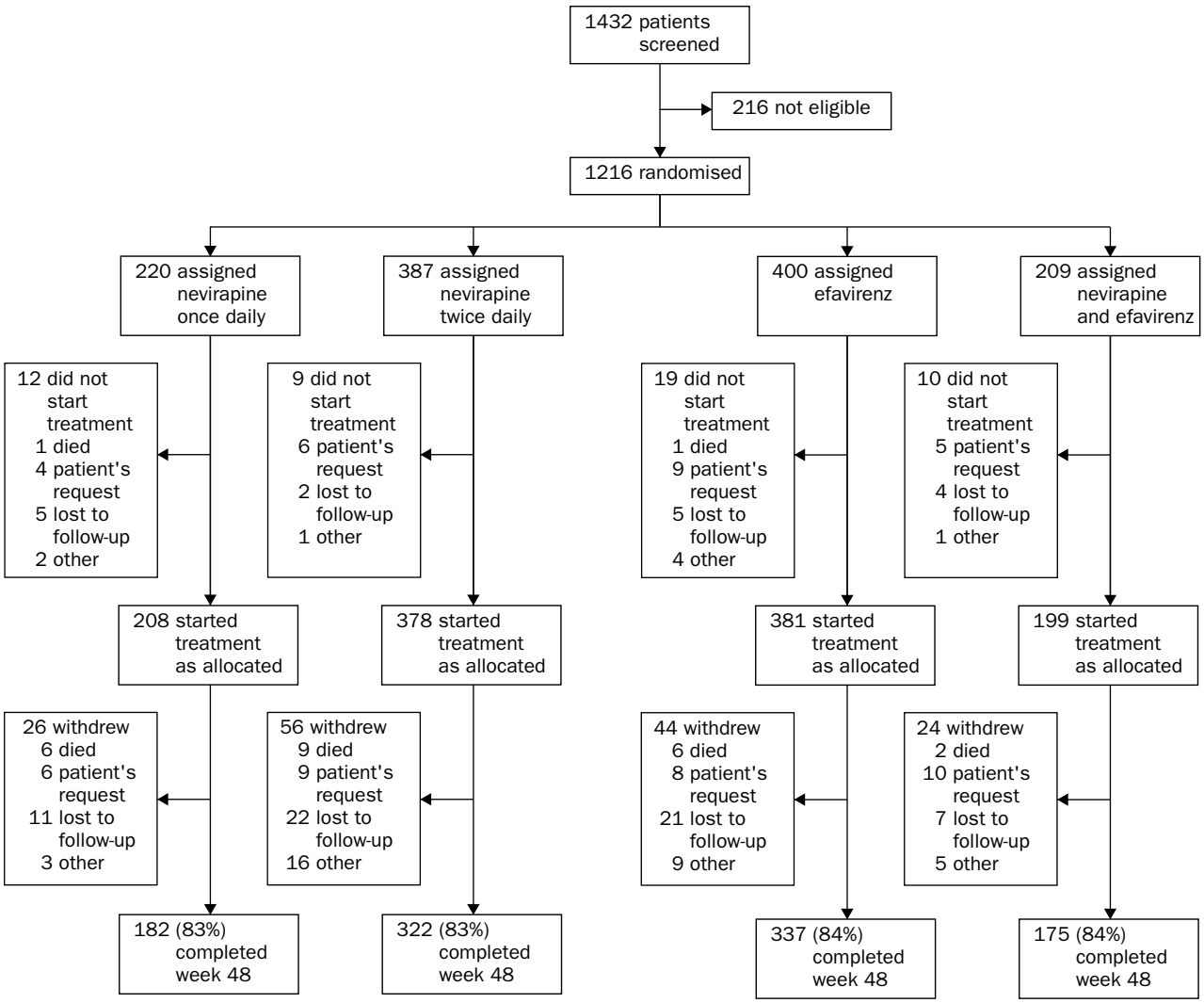


Figure 1: **Trial profile**
All patients received stavudine and lamivudine (see Methods). The reasons for exclusion were not recorded.

start of treatment and week 48; and the frequencies of clinical and laboratory adverse events. Matters that were reported as clinical adverse events but reflected only asymptomatic laboratory abnormalities were excluded from the clinical-adverse-events listing but incorporated in the listing of laboratory abnormalities. Laboratory abnormalities were classified as hepatic or non-hepatic. Since hepatic abnormalities were expected, this approach allowed us to examine the overall frequency of laboratory toxicities among the study groups apart from those associated with the liver. Hepatic laboratory abnormalities included an increase in aspartate or alanine amino-transferase and a high bilirubin concentration that was associated with an increase in these enzymes. Isolated rises in γ-glutamyl transferase were excluded because they reflect only enzyme induction caused by the use of nevirapine, efavirenz, or both. If an event occurred more than once in the same patient, that with the highest toxicity grade was included. All the analyses compared the treatment groups at week 48.

Statistical analyses

Categorical variables were tested with a χ^2 or Fisher's exact test, as appropriate. Continuous variables were tested with a Kruskal-Wallis test. Missing data on plasma HIV-1 RNA concentration were taken to be above 50 copies per mL. Patients who withdrew prematurely were therefore classified as failures because two measurements of plasma HIV-1 RNA concentration were missing and taken to be above 50 copies per mL. Risk factors for treatment failure were assessed by logistic regression.

All analyses were done for the intention-to-treat population, including all randomised patients (n=1216). In the safety analyses, only adverse events that occurred while the patient was taking the allocated treatment were included. A two-sided p value of less than 0·05 was deemed significant in comparisons of all four study groups (reported as "overall").

All outcome measurements were analysed by randomisation period (before or after addition of the fourth study group). The data for both periods were pooled only when the outcome was not modified by the randomisation period (non-significant interaction term in models excluding the group assigned nevirapine twice daily).

At the time that the fourth treatment group was added, four pairwise comparisons were defined: nevirapine once daily versus nevirapine twice daily; nevirapine twice daily versus efavirenz; efavirenz versus nevirapine plus efavirenz; and nevirapine once daily versus nevirapine plus efavirenz. This last analysis was chosen because

	Nevirapine once daily (n=220)	Nevirapine twice daily (n=387)	Efavirenz (n=400)	Nevirapine plus efavirenz (n=209)
Demographic and anthropometric characteristics				
M/F	139 (63·2%)/81 (36·8%)	236 (61·0%)/151 (39·0%)	254 (63·5)/146 (36·5%)	143 (68·4)/66 (31·6%)
Age, years*	34·4 (28·4–40·3)	33·9 (29·7–41·0)	34·7 (30·1–39·7)	33·2 (29·0–39·0)
Body-mass index, kg/m²*	19·2 (17·4–21·2)	19·7 (17·7–22·4)	19·3 (17·3–21·8)	19·2 (17·2–21·4)
Geographical region				
Asia/Australia (n=223)	52 (23·6%)	52 (13·4%)	76 (19·0%)	43 (20·6%)
Europe (n=249)	50 (22·7%)	72 (18·6%)	78 (19·5%)	49 (23·4%)
South Africa (n=430)	72 (32·7%)	146 (37·7%)	141 (35·2%)	71 (34·0%)
South America (n=249)	40 (18·2%)	89 (23·0%)	84 (21·0%)	36 (17·2%)
North America (n=65)	6 (2·7%)	28 (7·2%)	21 (5·3%)	10 (4·8%)
HIV risk behaviour				
Heterosexual	124 (56·4%)	236 (61·0%)	224 (56·0%)	111 (53·1%)
Homosexual	58 (26·4%)	102 (26·4%)	117 (29·3%)	73 (34·9%)
Intravenous drug use	11 (5·0%)	13 (3·4%)	21 (5·3%)	10 (4·8%)
Other, unknown	27 (12·2%)	36 (9·2%)	38 (9·5%)	15 (7·2%)
CDC class C	44 (20·0%)	86 (22·2%)	84 (21·0%)	39 (18·7%)
CD4-positive cells				
Median count, cells per μL*	200 (95–340)	170 (60–310)	190 (80–350)	190 (80–330)
Number <50 per μL	35 (15·9%)	79 (20·4%)	70 (17·5%)	28 (13·4%)
Number 50–200 per μL	76 (34·5%)	138 (35·7%)	144 (36·0%)	80 (38·3%)
Number >200 per μL	109 (49·5%)	170 (43·9%)	186 (46·5%)	101 (48·3%)
HIV-1 RNA				
Median $\log_{10}$ copies per mL*	4·7 (4·4–5·4)	4·7 (4·4–5·5)	4·7 (4·4–5·5)	4·7 (4·4–5·5)
Number <100 000	152 (69·1%)	264 (68·2%)	263 (65·8%)	139 (66·5%)
Number >100 000	68 (30·9%)	123 (31·8%)	137 (34·3%)	70 (33·5%)
Coinfection				
Hepatitis B virus†	15 (6·8%)	17 (4·4%)	16 (4·0%)	16 (7·7%)
Hepatitis C virus‡	22 (10·0%)	35 (9·0%)	40 (10·0%)	19 (9·1%)

CDC=Centers for Disease Control and Prevention. Data are number of patients unless indicated otherwise.*Median (IQR). †Presence of HBsAg at screening. ‡Presence of antibodies to hepatitis C virus.

Table 1: **Baseline characteristics of all randomised patients (n=1216)**

nevirapine was dosed once daily in the group assigned nevirapine plus efavirenz. On the basis of these four comparisons, the definition of significance was adjusted to $p<0·0125$ (Bonferroni's method). This significance definition was used for all pairwise comparisons, except for the primary outcome comparing nevirapine twice daily versus efavirenz. For that comparison we assessed equivalence of the two groups, with a null hypothesis of at least 10% more treatment failures with nevirapine twice daily than with efavirenz. Equivalence was assumed if the limits of the conventional 95% CI of this difference were within 10% of zero. The number of patients in the comparison (n=787) provided adequate power for assessment of equivalence within the 10% limits of the percentage of treatment failures if the two treatments were equivalent (assumed failure rate 35%, $\alpha=0·05$, $\beta=0·2$). A sensitivity analysis assessed equivalence in the same way with the exclusion of patients who never started allocated treatment. Data were analysed with SAS statistical software (version 8.02).

Two interim analyses were done. The first, at week 8, included the first 400 patients who were assigned nevirapine once daily, efavirenz, or nevirapine plus efavirenz. The purpose of this analysis was to assess viral decay within the first 2 weeks of treatment, to reassure the Data Safety and Monitoring Board that the novel combination of nevirapine and efavirenz did not underperform with respect to viral suppression. The second interim analysis was for all patients at week 24, to assess efficacy and safety of the four treatments. Results were shared only between the trial statistician and the Data Safety and Monitoring Board. The Board did not recommend discontinuation or modification of the trial at any stage.

Role of funding sources

The study was investigator initiated and financially supported by Boehringer-Ingelheim. The sponsor had a non-binding input on issues of study design and analyses, which did not lead to any significant influence on the

	Before protocol change				After protocol change			
	Nevirapine once daily (n=131)	Efavirenz (n=131)	Nevirapine plus efavirenz (n=126)	p	Nevirapine once daily (n=89)	Efavirenz (n=269)	Nevirapine plus efavirenz (n=83)	p
Treatment failure (%; 95% CI)	61 (46·6%; 37·8–55·5)	45 (34·4%; 26·3–43·2)	65 (51·6%; 42·5–60·6)	0·016	35 (39·3%; 29·1–50·3)	106 (39·4%; 33·5–45·5)	46 (55·4%; 44·1–66·3)	0·0289
Component of treatment failure								
Virological	16 (12·2%)	19 (14·5%)	18 (14·3%)	0·838	9 (10·1%)	42 (15·6%)	16 (19·3%)	0·235
Progression	5 (3·8%)	2 (1·5%)	3 (2·4%)	0·529	2 (2·3%)	8 (3·0%)	2 (2·4%)	0·918
Change of assigned treatment	40 (30·5%)	24 (18·3%)	44 (34·9%)	0·009	24 (27·0%)	56 (20·8%)	28 (33·7%)	0·048
HIV-1 RNA <50 copies per mL (%; 95% CI)	91 (69·5%; 60·8–77·2)	97 (74·1%; 65·7–81·3)	78 (61·9%; 52·8–70·4)	0·107	63 (70·8%; 60·2–80·0)	183 (68·0%; 62·1–73·6)	53 (63·9%; 52·6–74·1)	0·618
Median (IQR) CD4-cell increase, cells per μL	160 (105–230)	150 (90–230)	145 (70–240)	0·676	185 (100–320)	170 (70–260)	180 (100–290)	0·558

Data are number of pateints unless otherwise stated.

Table 2: **Outcome of efficacy analyses before and after protocol change in randomisation**

	A: Nevirapine once daily (n=220)	B: Nevirapine twice daily (n=387)	C: Efavirenz (n=400)	D: Nevirapine plus efavirenz (n=209)	p*				
					Overall	A vs B	B vs C	A vs D	C vs D
Treatment failure on or before week 48 (%; 95% CI)	96 (43·6%; 37·0–50·5)	169 (43·7%; 38·7–48·8)	151 (37·8%; 33·0–42·7)	111 (53·1%; 46·1–60·0)	0·004	0·994	0·091	0·050	0·0003
Components of failure									
Virological	25 (11·4%)	73 (18·9%)	61 (15·3%)	34 (16·3%)	0·108	0·016	0·178	0·140	0·742
Progression	7 (3·2%)	11 (2·8%)	10 (2·5%)	5 (2·4%)	0·949	0·813	0·766	0·620	0·935
Change of treatment	64 (29·1%)	85 (22·0%)	80 (20·0%)	72 (34·4%)	0·0002	0·050	0·499	0·233	<0·0001
Permanent change of NNRTI	37	61	51	51					
Temporary discontinuation of NNRTI	13	13	8	6					
Additional antiretroviral drugs	2	1	1	2					
Non-allowable change of NRTI	0	1	1	3					
Never started ART	12	9	19	10					
Plasma HIV-1 RNA concentration <50 copies per mL at 48 weeks (%; 95% CI)†	154 (70·0%; 63·5–76·0)	253 (65·4%; 60·4–70·1)	280 (70·0%; 65·2–74·5)	131 (62·7%; 55·7–69·3)	0·193	0·244	0·165	0·109	0·067

ART=antiretroviral therapy. *For significance: overall (four groups) p<0·05, pairwise comparisons p<0·0125. †Intention-to-treat population.

Table 3: **Number of patients with treatment failure, components of treatment failure, and number of patients with plasma HIV-1 RNA concentration <50 copies per mL**

resulting design and analyses. The sponsor was allowed to provide comments on the report in progress but had no influence on reporting of the data or the decision to publish. All investigators and the sponsor had full access to the data after official closure of the database.

Results

Of 1432 patients screened, 1216 underwent randomisation between February, 2000, and June, 2001. 5 months after the start of enrolment, a group to receive nevirapine twice daily was opened for treatment allocation as described above. 50 patients, who potentially knew their treatment allocation, never started their study drugs. In total, 1166 patients started follow-up, of whom 981 (81% of those randomised) reached week 48 (figure 1). The baseline characteristics of all randomised patients were similar among the study groups (table 1).

Table 2 summarises the results from the efficacy analyses separately for the periods before and after the randomisation change. In both periods, the proportion of patients with treatment failure differed significantly among the treatment groups and was highest in the group assigned nevirapine plus efavirenz. In both randomisation periods, the main reason for treatment failure was change of allocated treatment, which occurred in a higher proportion of the nevirapine plus efavirenz group than of the other groups. In the logistic regression analysis to assess the effect of randomisation period on the risk of treatment failure, there was no evidence for such an effect (odds ratio 1·01, p=0·868), nor significant evidence that treatment differences varied by randomisation period (interaction term p=0·326). Furthermore, there were no significant effects of randomisation period on the proportion of patients with plasma HIV-1 RNA concentrations below 50 copies per mL at week 48 or the increase in CD4-positive T cells (interaction p=0·513 and 0·892, respectively). Subsequently, the data were pooled for all efficacy analyses, despite numerical differences between the randomisation periods.

The results of the primary analysis and the contributions of the three components to the composite endpoint for all patients are summarised in table 3. The proportion of patients with treatment failure at week 48 was 43·6% (95% CI 37·0 to 50·5) for nevirapine once daily, 43·7% (38·7 to 48·8) for nevirapine twice daily, 37·8% (33·0 to 42·7) for efavirenz, and 53·1% (46·1 to 60·0) for nevirapine plus efavirenz (overall p=0·004). The difference of 5·9% (95% CI –0·9 to 12·8) between the groups assigned nevirapine twice daily or efavirenz was not significant (p=0·091), but equivalence within the 10% limits could not be demonstrated.

The sensitivity analysis testing for equivalence of nevirapine twice daily and efavirenz in the population after exclusion of patients who never started treatment showed similar results to the primary analysis (7·7% [95% CI 0·8 to 14·6], p=0·03). In the population of concurrently randomised patients (including only those randomised after the protocol change) the difference was 4·3% (–3·4 to 11·9, p=0·276).

There was no significant difference between patients assigned nevirapine once daily and those assigned nevirapine twice daily (0·1%, p=0·994). Patients assigned nevirapine plus efavirenz had a significantly higher probability of treatment failure than those assigned efavirenz (15·3%, p=0·0003) but no significant difference from those assigned nevirapine once daily (9·5%, p=0·05).

	Nevirapine once daily	Nevirapine twice daily	Efavirenz	Nevirapine plus efavirenz	Nevirapine twice daily vs efavirenz (95% CI)
Treatment failure					
Asia/Australia	23 (44·2%)	17 (32·7%)	15 (19·7%)	24 (55·8%)	
South Africa	33 (45·8%)	73 (50·0%)	54 (38·3%)	35 (49·3%)	5·9 (–0·9 to 12·8)*
South America	15 (37·5%)	35 (39·3%)	33 (39·3%)	14 (38·9%)	
Europe/USA/Canada	25 (44·6%)	44 (44·0%)	49 (49·5%)	38 (64·4%)	
Asia/Australia	23 (44·2%)	17 (32·7%)	15 (19·7%)	24 (55·8%)	13·0 (–2·6 to 28·5)
Other	73 (43·5%)	152 (45·4%)	136 (42·0%)	87 (52·4%)	3·4 (–4·2 to 11·0)
Virological failure					
Asia/Australia	11 (21·2%)	13 (25·0%)	13 (17·1%)	9 (20·9%)	
South Africa	31 (43·1%)	65 (44·5%)	49 (34·8%)	31 (43·7%)	
South America	9 (22·5%)	26 (29·2%)	25 (29·8%)	9 (25·0%)	
Europe/USA/Canada	26 (46·4%)	37 (37·0%)	42 (42·4%)	31 (52·5%)	

Percentages are for the total of patients per group per region. *For overall trial, all four regions.

Table 4: **Numbers of patients with treatment failure or virological failure by region**

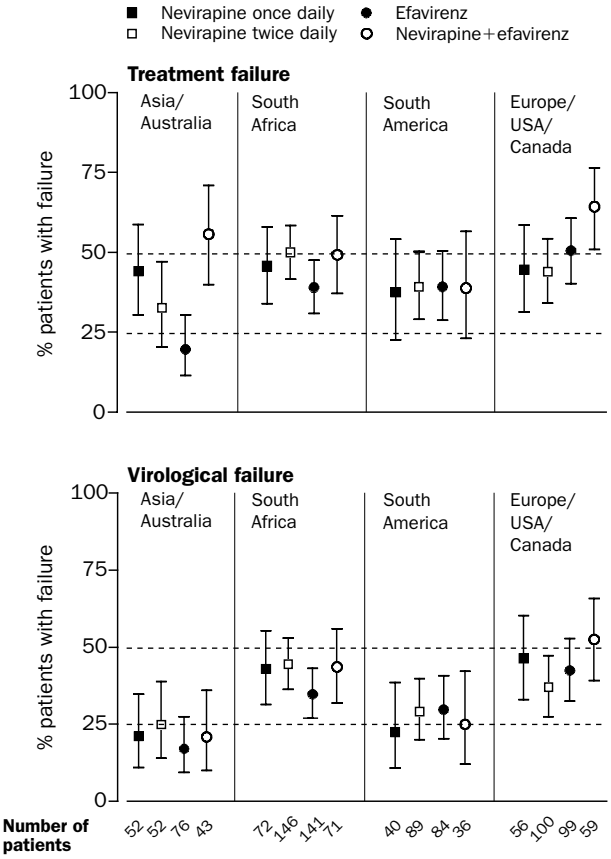


Figure 2: **Proportion of patients with treatment failure or virological failure by region**
Error bars=95% CI.

The main reason for treatment failure in each of the study groups was change of allocated treatment (table 3). The only difference significant at p=0·0125 was that between efavirenz and nevirapine plus efavirenz (14·4%, p<0·0001). The change of allocated treatment in all groups was predominantly a permanent change in the NNRTI component.

The response to treatment differed among the geographical regions (interaction between region and treatment group p=0·035). In Asia and Australia (90% of the patients in this region were enrolled at one centre in Bangkok, Thailand), the proportion of treatment failure in the efavirenz group was much lower and the differences between the nevirapine groups larger than in the other regions (table 4, figure 2). There were no significant differences between the regions other than Asia and Australia (interaction term p=0·328). Despite this modifying effect of region, testing for equivalence of nevirapine twice daily and efavirenz within the 10% limits gave similar results to the primary analysis: equivalence could not be established in either Asia and Australia or the other regions combined (table 4). However, this analysis lacks power and should be interpreted cautiously.

For virological failure, there was no significant interaction between outcome and region (interaction term p=0·629; table 4, figure 2), but region remained significant with Asia and Australia having virological failure rates below 25% and Europe, the USA, and Canada having rates approaching 50%.

The proportion of patients reaching a plasma HIV-1 RNA concentration of less than 50 copies per mL at week 48 is summarised in table 2 for each randomisation period and in figure 3 for all patients. The mean values at week 48 were 70·0% (95% CI 63·5 to 76·0) for nevirapine once daily, 65·4% (60·4 to 70·1) for nevirapine twice daily, 70·0% (65·2 to 74·5) for efavirenz, and 62·7% (55·7 to 69·3) for nevirapine plus efavirenz. Overall, there were no significant differences in any of the four pairwise comparisons.

The treatment groups had similar median increases in CD4-positive T cells over 48 weeks (overall p=0·8). The median increase was 170 cells per μL for nevirapine once daily, 160 cells per μL for the groups assigned nevirapine twice daily and efavirenz, and 150 cells per μL for nevirapine plus efavirenz (figure 4). Age, sex, disease stage, baseline CD4-positive cell count, and mode of HIV-1 transmission were not associated with treatment failure.

Patients with a baseline plasma HIV-1 RNA concentration of more than 100 000 copies per mL were 1·63 times (95% CI 1·28 to 2·07) more likely than those with baseline concentrations below this value to experience treatment failure. The difference in treatment failure rate between patients with high and low baseline plasma HIV-1 RNA concentrations was 19·9% for

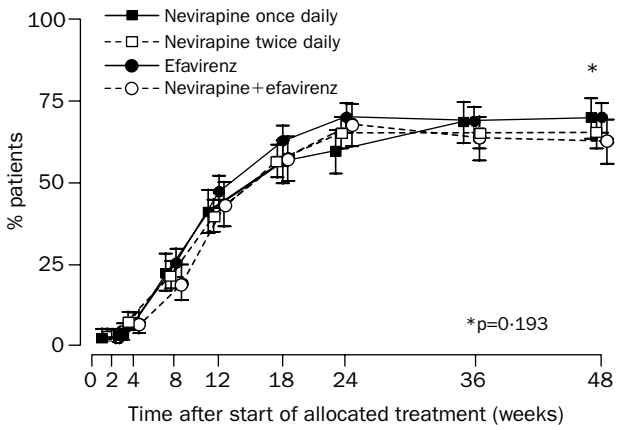
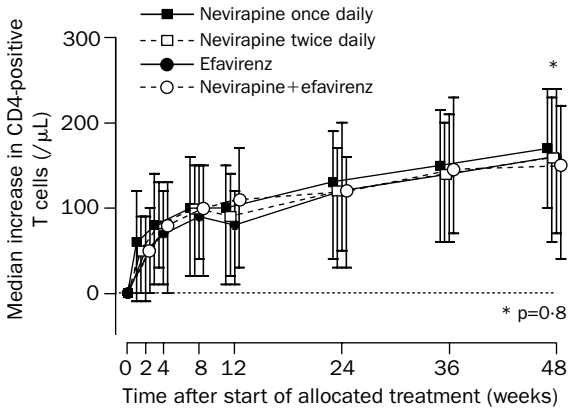


Figure 3: **Proportion of patients with plasma HIV-1 RNA concentrations below 50 copies per mL**
Error bars=95% CI.



Number of patients with counts

Nevirapine once daily	208	189/186/181	179	180	174
Nevirapine twice daily	376	349/329/337	323	313	313
Efavirenz	381	360/351/351	343	330	326
Nevirapine+ efavirenz	198	185/174/177	175	164	168

Figure 4: **CD4-positive T-cell response over 48 weeks**
Dotted line represents the baseline value. Error bars=IQR.

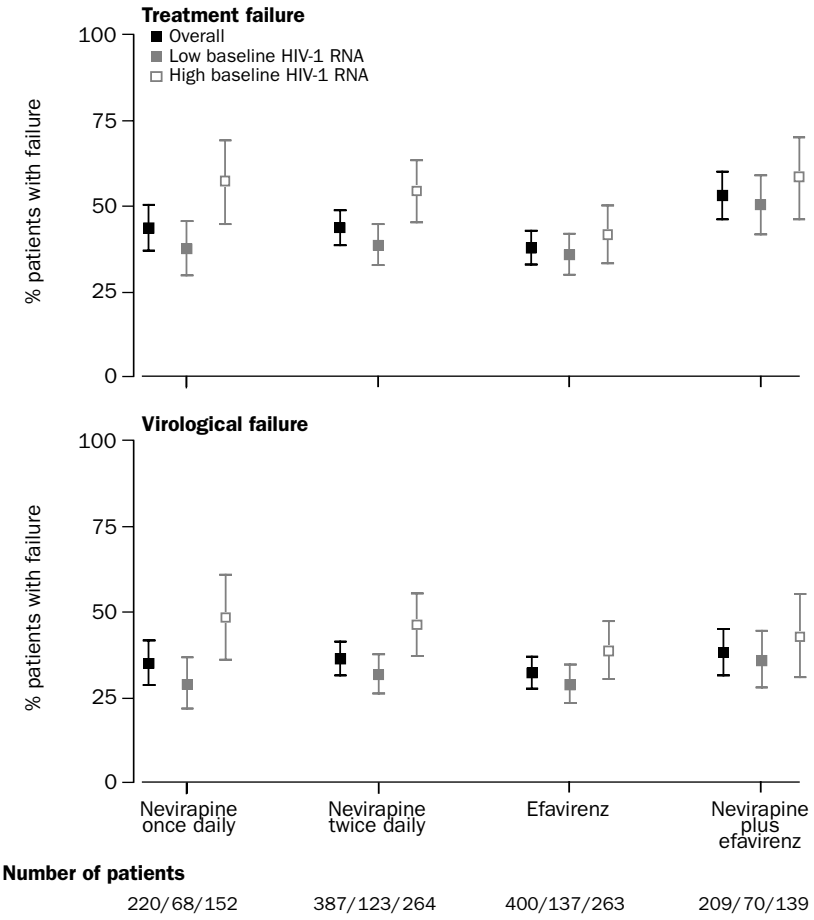


Figure 5: **Proportion of patients with treatment failure or virological failure by baseline plasma HIV-1 RNA concentration**

Overall=all patients randomised. Low baseline=patients with baseline plasma HIV-1 RNA ≤100 000 copies/mL. High baseline=patients with baseline plasma HIV-1 RNA concentration >100 000 copies/mL. Error bars=95% CI.

nevirapine once daily, 15·8% for nevirapine twice daily, 5·9% for efavirenz, and 8·2% for nevirapine plus efavirenz (overall p=0·004). However, the adverse effect of high baseline plasma HIV-1 RNA concentration on the probability of treatment failure was similar for all treatment groups (interaction term p=0·368; figure 5). If the groups assigned nevirapine only were combined, the difference in treatment failure within this stratum was 17·3%. Again, the adverse effect of a high baseline plasma HIV-1 RNA concentration on treatment failure did not differ between the combined nevirapine group and the efavirenz group (interaction term p=0·105). The same effect of high baseline plasma HIV-1 RNA concentration was seen for virological failure (interaction term p=0·571; figure 5).

In total, 25 patients died during the study, two before taking any study medication. Seven (3·2%) patients assigned nevirapine once daily died, compared with nine (2·3%) assigned nevirapine twice daily, seven (1·8%) assigned efavirenz, and two (1·0%) assigned nevirapine plus efavirenz.

One patient assigned nevirapine twice daily, without evidence of hepatitis B or C virus coinfection, developed fulminant hepatitis, attributed to the use of nevirapine, as well as pancreatitis and renal failure 32 days after the start of treatment; this patient died a week later. Another patient assigned nevirapine twice daily developed Stevens-Johnson syndrome, attributed to the use of nevirapine, 39 days after the start of treatment; although recovering, she was admitted to hospital for assessment of blepharitis. While in hospital she developed septicaemia due to a meticillin-resistant *Staphylococcus aureus* infection and died after 8 days. One patient assigned nevirapine once daily died of lactic acidosis that was attributed to the use of stavudine.

11 deaths were related to HIV-1 disease: three were due to *Pneumocystis carinii* pneumonia, one each to generalised cytomegalovirus infection, tuberculous meningitis, *Mycobacterium avium* infection, generalised histoplasma infection, and Kaposi's sarcoma, and three to end-stage HIV-1 disease. The other 11 deaths were not related to study medication or HIV-1 disease (murder, renal failure, brainstem glioma, suicide of a patient who had not started study medication, congestive heart failure, myocardial infection [two], endocarditis, empyema, and unknown reason [two]).

For all reported safety analyses, the change in randomisation did not modify the outcome analysed.

	A: Nevirapine once daily (n=220)	B: Nevirapine twice daily (n=387)	C: Efavirenz (n=400)	D: Nevirapine plus efavirenz (n=209)	p*				
					Overall	A *vs* B	B *vs* C	A *vs* D	C *vs* D
Reasons for temporary or permanent discontinuation of study drugs									
Adverse events or HIV event	53 (24·1%)	83 (21·5%)	63 (15·8%)	63 (30·1%)	0·0005	0·453	0·040	0·158	<0·0001
Patient's request	5 (2·3%)	8 (2·1%)	15 (3·8%)	16 (7·7%)	0·004	0·877	0·161	0·01	0·037
Physician's decision	14 (6·4%)	26 (6·7%)	11 (2·3%)	20 (9·6%)	0·005†	0·866	0·009	0·219	<0·0001
Other	27 (12·3%)	32 (8·3%)	36 (9·0%)	27 (12·9%)	0·175	0·109	0·715	0·840	0·132
Adverse events leading to temporary or permanent discontinuation of study drugs									
Rash	27 (12·3%)	25 (6·5%)	15 (3·8%)	29 (13·9%)					
CNS/psychiatric	11 (5·0%)	22 (5·7%)	27 (6·7%)	16 (7·7%)					
Clinical hepatitis	4 (1·8%)	6 (1·6%)	1 (0·3%)	2 (1·0%)					
Peripheral neuropathy	6 (2·7%)	12 (3·1%)	9 (2·3%)	8 (3·8%)					
Hepatic laboratory abnormality	13 (5·9%)	9 (2·3%)	1 (0·3%)	6 (2·9%)					
Vomiting	1 (0·5%)	5 (1·3%)	5 (1·3%)	4 (1·9%)					

Data are number of patients. CNS=central nervous system.*For significance: overall (four groups) p<0·05, pairwise comparisons p<0·0125. †Fisher's exact test for overall significance and subsequent pairwise comparisons.

Table 5: **Reasons for temporary or permanent discontinuation of study drugs**

	A: Nevirapine once daily (n=220)	B: Nevirapine twice daily (n=387)	C: Efavirenz (n=400)	D: Nevirapine plus efavirenz (n=209)	p*				
					Overall	A *vs* B	B *vs* C	A *vs* D	C *vs* D
Clinical adverse events									
Hepatobiliary	4 (1·8%)	10 (2·6%)	2 (0·5%)	2 (1·0%)	0·082†	0·546	0·017	0·686	0·610
Clinical hepatitis	3 (1·4%)	8 (2·1%)	1 (0·3%)	2 (1·0%)					
Cutaneous	9 (4·1%)	14 (3·6%)	15 (3·8%)	12 (5·7%)	0·619	0·769	0·922	0·428	0·257
Rash	9 (4·1%)	13 (3·4%)	8 (2·0%)	11 (5·3%)					
CNS/psychiatric	3 (1·4%)	14 (3·6%)	22 (5·5%)	16 (7·7%)	0·001	0·106	0·337	0·002	0·296
Insomnia/abnormal dreams	0	0	6 (1·5%)	5 (2·4%)					
Anxiety	0	0	4 (1·0%)	3 (1·4%)					
Depression	0	1 (0·3%)	6 (1·5%)	1 (0·5%)					
Diarrhoea	1 (0·5%)	3 (0·8%)	4 (1·0%)	4 (1·9%)					
Vomiting	3 (1·4%)	4 (1·0%)	4 (1·0%)	3 (1·4%)					
Pyrexia	2 (0·9%)	8 (2·1%)	3 (0·8%)	2 (1·0%)					
Total number of patients‡	33 (15·0%)	79 (20·4%)	72 (18·0%)	51 (24·4%)	0·077	0·098	0·390	0·014	0·062
Laboratory toxicities									
Hepatobiliary	30 (13·6%)	32 (8·3%)	18 (4·5%)	19 (9·1%)	0·001	0·036	0·030	0·139	0·024
Non hepatobiliary	20 (9·1%)	53 (13·7%)	41 (10·3%)	22 (10·5%)	0·276	0·094	0·133	0·617	0·915
Neutropenia	6 (2·7%)	17 (3·9%)	9 (2·3%)	13 (6·2%)					
Amylase increased	4 (1·8%)	13 (3·6%)	15 (3·8%)	3 (1·4%)					
Triglycerides increased	3 (1·4%)	5 (1·3%)	5 (1·3%)	1 (0·5%)					
Alkaline phosphatase	2 (0·9%)	5 (1·3%)	3 (0·8%)	4 (1·9%)					

*For significance: overall (four groups) p<0·05, pairwise comparisons p<0·0125. †Fisher's exact test for overall significance and subsequent pairwise comparisons. ‡Number with at least one grade 3 or 4 clinical adverse event.

Table 6: **Numbers of patients with at least one grade 3 or 4 clinical adverse event and laboratory toxicities**

Therefore, all safety analyses are reported for the pooled data. The reasons for temporary or permanent discontinuation of one of the study drugs are summarised in table 5. The reasons are not mutually exclusive. The commonest reason for discontinuation was adverse events. The rate of adverse events differed significantly by study group. However, of the predefined comparisons, only the difference between efavirenz and nevirapine plus efavirenz was significant at p=0·0125 (14·3%, p<0·0001). The difference between nevirapine once daily and efavirenz also reached statistical significance (8·3%, p=0·011).

Although the randomisation period did not statistically modify the proportion of patients who temporarily or permanently discontinued study drugs because of an adverse event or HIV event (interaction p=0·106), patients assigned efavirenz were more likely to discontinue study drugs for this reason before the randomisation change than afterwards. However, this difference did not result in a significantly different proportion of patients in the efavirenz group having treatment failure because of change of allocated treatment (table 2).

15·0% of patients in the group assigned nevirapine once daily had at least one grade 3 or 4 clinical adverse event, compared with 20·4% of those assigned nevirapine twice daily, 18·0% of those assigned efavirenz, and 24·4% of those assigned nevirapine plus efavirenz (table 6; overall p=0·077). There were no statistically significant differences between the single NNRTI groups. The difference in frequency between the group assigned nevirapine plus efavirenz and the nevirapine once daily group was significant (p=0·014), but that between the group assigned nevirapine plus efavirenz and the efavirenz group was not (p=0·062). The grade 3 or 4 adverse events that were reported in more than 1% of the patients are listed in table 6.

The proportion of patients with at least one grade 3 or 4 liver-associated laboratory toxicity was 13·6% for nevirapine once daily, 8·3% for nevirapine twice daily, 4·5% for efavirenz, and 9·1% for nevirapine plus efavirenz (table 6; overall p=0·001). None of the predefined pairwise comparisons was significant, but the frequency was significantly higher for nevirapine once daily than for efavirenz (p<0·0001). Of the patients with grade 3 or 4 liver-associated laboratory toxicity, the proportions coinfected with hepatitis B or hepatitis C virus were 13·8% and 20·7% in the nevirapine once daily group, 6·7% and 20·0% in the nevirapine twice daily group, 5·6% and 11·1% in the efavirenz group, and 16·7% and 22·2% in the nevirapine plus efavirenz group. In the total study population, the rates of hepatitis B and C coinfection were 7·7% and 9·1%.

There were no significant differences between the groups in the incidence of non-hepatobiliary laboratory toxicities, overall or by randomisation period.

Discussion

For each randomisation period separately, and overall, we found a significant difference in the proportion of patients with treatment failure at week 48, which was driven by the higher risk of failure in the group assigned nevirapine plus efavirenz. Although, overall, treatment failure was numerically lower in the efavirenz group than in the nevirapine-only groups, our findings show no evidence that efavirenz is superior to nevirapine twice daily in terms of treatment failure. However, we could not show equivalence within the 10% limits of these treatment groups even though the study was adequately powered for such an analysis. The performance of nevirapine twice daily compared favourably with that of other drugs that have been formally compared with efavirenz. In previous studies, efavirenz was found to have better efficacy than indinavir,[1] nelfinavir,[16] or protease-inhibitor-based regimens in general.[17] Nevirapine once or twice daily showed similar rates of treatment failure in our study, which accords with findings by others in small studies.[18,19] The simultaneous use of nevirapine and efavirenz resulted in a higher probability of treatment failure, mainly due to increased rates of change of allocated treatment because of an adverse event.

There were no significant differences between the study groups in the proportion of patients with virological failure, the proportion with plasma HIV-1 RNA concentrations below 50 copies per mL at week 48, or the increase in CD4-positive T cells during the study. The difference in treatment failure rates between patients with baseline plasma HIV-1 RNA concentrations above and below 100 000 copies per mL was largest for the

nevirapine-only groups, but a detrimental effect of high baseline plasma HIV-1 RNA concentration was observed for all regimens. A higher probability of treatment or virological failure in patients with such a high baseline viral load has been described previously for nevirapine,[20,21] but not for efavirenz.[1]

The observed regional differences in treatment failure might be attributable to physicians' management of patients within the study (210 of the 223 patients in Asia and Australia region were from a single treatment centre in Bangkok). Another reason might be population differences in frequencies of drug-related adverse events, since in Asia and Australia 26% of patients temporarily or permanently discontinued study treatment because of adverse events, whereas in other regions this proportion was between 18% and 23%. However, distinction between these two hypotheses is difficult, and they are not mutually exclusive. The high rate of discontinuation in Asia and Australia might also be the cause of the difference in temporary or permanent discontinuation of study drug in the efavirenz group in the comparison of both randomisation periods. In the period before randomisation change there was over-representation of patients from Asia and Australia.

Owing to the randomisation change and the regional differences, the presented overall efficacy estimates should be interpreted with caution. However, we believe that the main conclusions of the study are robust. The contributions of the reasons for treatment failure are similar in the two randomisation periods. Furthermore, the difference in treatment failure in both periods is driven by the poor performance of nevirapine plus efavirenz. The secondary efficacy endpoints are also similar in the two periods. With respect to the effect of region on treatment failure, the conclusions about the comparisons of nevirapine twice daily versus efavirenz, and nevirapine once daily versus nevirapine twice daily, as well as the poor performance of the nevirapine plus efavirenz group can be drawn for the region Asia and Australia alone as well as for the other regions combined. Thus, the results of this study can be seen as qualitatively generalisable in terms of the direction of effects observed, but not numerically generalisable in terms of the size of the efficacy estimates.

Our findings contrast with those of cohort studies in antiretroviral-therapy-naive patients. In the Italian ICoNA cohort, patients who started treatment with two NRTIs and nevirapine had a relative hazard of virological failure of 2·15 (95% CI 0·9 to 5·1) compared with those starting treatment with two NRTIs and efavirenz.[7] Although this estimate did not achieve conventional statistical significance, the increased risk is large. In a cohort of 888 patients, Matthews and colleagues found a hazard ratio for therapy failure at 24 weeks of 2·03 (1·26 to 3·18) for patients starting nevirapine-based antiretroviral therapy compared with those starting an efavirenz-based therapy.[9] In a study by Keiser and co-workers,[8] including more than 1000 patients, efavirenz was superior to nevirapine in terms of time to virological failure, decrease in plasma HIV-1 RNA concentration, and the proportion of patients with undetectable plasma HIV-1 RNA.

The only data from a randomised trial were reported by Núñez and colleagues.[22] In this small trial, 64 therapy-naive patients started a regimen of stavudine and didanosine with either nevirapine or efavirenz; 64% of the nevirapine group and 74% of the efavirenz group had plasma HIV-1 RNA concentrations below 50 copies per mL after 48 weeks in an intention-to-treat analysis (p=0·43). However, the study had power only of 14% to find a significant difference in this variable.

The disparities between results from cohort studies and the 2NN Study might be partly explained by differences in study design. Since there is no randomisation in cohort studies, selection bias could have been introduced if the choice between nevirapine and efavirenz were influenced by patients and physicians. Physicians' preconceived ideas about efficacy and safety of certain therapy regimens will have a role in decisions on a therapy regimen for a particular patient and on management. Knowledge and experience of physicians is clearly associated with the adaptation of new treatment strategies.[23] Although these biases can be kept to a minimum by the use of multivariate models,[24] the possibility of residual confounding in cohort studies remains. However, differences in management of patients in an open-label clinical trial such as the 2NN, even when within the boundaries of the study protocol, could also lead to biased estimates. The magnitude of such bias in clinical trials is difficult to establish but it should not be ignored, especially when the chosen composite endpoint includes a component that is easily influenced by management of patients.

The two nevirapine-attributed deaths were caused by known complications of the drug. Strict adherence to guidelines on management of rashes could minimise the risk of Stevens-Johnson syndrome.[25,26] However, monitoring of plasma aminotransferase concentrations might not prevent the occurrence of fulminant hepatitis.[27,28]

The reported frequency of clinical and laboratory adverse events in patients taking nevirapine or efavirenz varies widely. We reported all grade 3 or 4 events irrespective of the relation of the event to the study drugs, to minimise ascertainment bias in the reporting of events, although the open-label design of the study cannot fully prevent the occurrence of such bias. The frequencies of grade 3 or 4 rash in the nevirapine once daily and twice daily groups (4·1% and 3·4%) were at the lower end of the range reported in other studies. In the ATLANTIC study, 7% of patients assigned stavudine, didanosine, and nevirapine developed a grade 3 or 4 rash.[2] In the INCAS study, severe rash (grade 4) was seen in 4% of patients allocated zidovudine plus nevirapine with or without didanosine.[29] Pollard and co-workers reported a grade 3 or 4 rash in 6·6% of the patients included in clinical trials.[30]

Grade 3 or 4 adverse events related to the central nervous system were seen only in study groups assigned efavirenz, apart from one patient assigned nevirapine twice daily who had depression. Staszewski and colleagues reported that 58% of patients allocated zidovudine, lamivudine, and efavirenz had some kind of central nervous system symptom, although none of the events was reported to be severe.[1]

The frequencies of hepatic laboratory abnormalities and clinical hepatotoxicity were similar to those reported previously. Patients assigned nevirapine once daily had a higher frequency of hepatic laboratory abnormalities than those assigned this drug twice daily. Peak plasma concentrations of nevirapine are greater with once daily than with twice daily dosing, but the total exposure to the drug is nearly identical.[31] Previous studies have not confirmed a relation between nevirapine concentration and toxicity.[31,32]

The occurrence of liver-associated laboratory toxicities is known to be increased in patients coinfected with hepatitis B or hepatitis C virus.[33,34] The proportions of patients with hepatic coinfection who developed liver-associated laboratory toxicity differed between the groups, but the numbers of patients were too small to allow

definitive conclusions about the effect of hepatic coinfection on the risk of such effects. Factors other than hepatic coinfection could be in play.

Apart from the drawback of the change in randomisation, the 2NN study has the limitation of an open-label design, which might have influenced decisions to change therapy. Such influences could affect the result for the composite endpoint, which was driven by the change-of-treatment component. However, the study was based in centres that had extensive experience with antiretroviral therapies. Inappropriate treatment changes are unlikely to have occurred on a scale large enough to render the study results not reproducible. Virological and immunological endpoints based on laboratory results are less influenced by the open-label design. These endpoints support the findings of the composite endpoint in that there were no significant differences among the single NNRTI groups of the 2NN Study.

The follow-up of 48 weeks in this study does not differ from that in many other HIV trials but is shorter than in cohort studies. There is a possibility that the difference between nevirapine twice daily and efavirenz will grow larger over time and that the efficacy of efavirenz is more sustainable than that of nevirapine twice daily.[35] Further follow-up of 2NN patients is planned. The overall drop-out rate of less than 20% is similar for all study groups, and similar to other large HIV clinical trials.

We conclude that the 2NN Study showed no significant differences in treatment failure between regimens with a single NNRTI in the treatment of antiretroviral-therapy-naive HIV-1-infected patients. Although nevirapine twice daily and efavirenz were not equivalent within the 10% limit, the two regimens are valid options for first-line therapy. However, the use of nevirapine was associated with two deaths in the study. The safety profiles of the drugs differ and should be taken into account when regimens are being chosen. Nevirapine once daily had a similar antiretroviral efficacy to nevirapine twice daily and seems therefore suitable for treatment simplification. The regimen of nevirapine plus efavirenz did not have additional efficacy and caused more adverse events than each drug separately. This regimen is therefore less desirable than the other treatment options presented in this study.

Contributors

J M A Lange and R van Leeuwen wrote the first draft of the study protocol. All members of the Steering Committee (J M A Lange [chair], K Ruxrungtham, B Gazzard, P Cahn, C Katlama, R L Murphy, A Horban, R Wood, and R van Leeuwen) contributed to the writing of the study protocol. F van Leth and E Hassink did all statistical analyses. J P Dam was the project manager and responsible for all data management at IATEC. All investigators were responsible for implementation of the study, management of patients, and data collection at the study sites, and had the opportunity to comment on the report. F van Leth, E Hassink, and F W Wit wrote the first draft of the report, and all members of the writing committee (F van Leth, P Phanuphak, B Gazzard, P Cahn, F Raffi, C Katlama, P Robinson, F W Wit, R van Leeuwen, J M A Lange) contributed at subsequent stages.

Conflict of interest statement

F van Leth has received travel grants and honoraria for presentations at symposia from Boehringer-Ingelheim. P Phanuphak has received honoraria from Bristol-Myers-Squibb as a scientific consultant and research grants from Bristol-Myers-Squibb, Hoffmann-LaRoche, GlaxoSmithKline, and Merck, Sharp and Dohme. K Ruxrungtham has received travel grants, grants, consultancy fees, and honoraria from various pharmaceutical companies including Hoffmann-LaRoche, Merck, Sharp and Dohme, Bristol-Myers-Squibb, and Abbott. S Miller has received consultancy fees from Bristol-Myers-Squibb and honoraria for presentations from Abbott and Boehringer-Ingelheim. B Gazzard has received travel grants, research grants, honoraria, and consultancy fees from various pharmaceutical companies including Boehringer-Ingelheim, Bristol-Myers-Squibb, GlaxoSmithKline, and Abbott Pharmaceuticals. P Cahn has received travel grants, grants, consultancy fees, and honoraria from various pharmaceutical companies including Shire, GlaxoSmithKline, Abbott, and Serono; he also has served as a consultant for Abbott and GlaxoSmithKline. I P van der Westhuizen has received consultancy fees from pharmaceutical companies. M A Johnson has received consultancy fees and lecture fees for presentations from Abbott, Bristol-Myers-Squibb, Hoffmann-LaRoche, and GlaxoSmithKline, and consultancy fees from Tibotec. F Mulcahy has received travel grants from GlaxoSmithKline and travel grants and honoraria for presentations at workshops from Bristol-Myers-Squibb, and he has also served as a consultant on the Advisory Board of Boehringer-Ingelheim. G C Levi has received lecture fees for presentations from Bristol-Myers-Squibb. G Reboredo has received travel grants and consultancy fees from various pharmaceutical companies including Bristol-Myers-Squibb, GlaxoSmithKline, and Hoffmann-LaRoche. K Squires has received consultancy fees and lecture fees for presentations from Gilead Science, Boehringer-Ingelheim, GlaxoSmithKline, and Abbott, and consultancy fees from Merck, Sharp and Dohme and Tibotec. F Raffi has received travel grants, grants, consultancy fees, and honoraria from various pharmaceutical companies including Abbott, Boehringer-Ingelheim, Bristol-Myers-Squibb, GlaxoSmithKline, and Hoffmann-LaRoche. C Katlama has received travel grants, grants, consultancy fees, and honoraria for presentations from Bristol-Myers-Squibb, GlaxoSmithKline, Boehringer-Ingelheim, Bayer, and Hoffmann-LaRoche. R Murphy has received consultancy fees from Boehringer-Ingelheim and Bristol-Myers-Squibb Pharmaceuticals. R van Leeuwen has received honoraria from various pharmaceutical companies including Boehringer-Ingelheim and Hoffmann-LaRoche. P Robinson is an employee of Boehringer-Ingelheim Pharmaceuticals company. F W Wit has received travel grants from Boehringer-Ingelheim, Abbott, Hoffmann-LaRoche, and GlaxoSmithKline, and honoraria for symposia from Boehringer-Ingelheim. J M A Lange has received consultancy fees and honoraria from GlaxoSmithKline, Boehringer-Ingelheim, Bristol-Myers-Squibb, Hoffmann-LaRoche, Merck, Sharp and Dohme, Schering-Plough, Bayer, Shire Pharmaceuticals, Agouron/Pfizer, and Virco/Tibotec.

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Cost-Effectiveness of Highly Active Antiretroviral Therapy in South Africa

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Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HAART, highly active antiretroviral therapy; IQR, interquartile range; LYG, life-year gained; OR, odds ratio; PPY, per patient-year; WHO, World Health Organization

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ABSTRACT

Background

Little information exists on the impact of highly active antiretroviral therapy (HAART) on health-care provision in South Africa despite increasing scale-up of access to HAART and gradual reduction in HAART prices.

Methods and Findings

Use and cost of services for 265 HIV-infected adults without AIDS (World Health Organization [WHO] stage 1, 2, or 3) and 27 with AIDS (WHO stage 4) receiving HAART between 1995 and 2000 in Cape Town were compared with HIV infected controls matched for baseline WHO stage, CD4 count, age, and socioeconomic status, who did not receive antiretroviral therapy (ART; No-ART group). Costs of service provision (January 2004 prices, US$1 = 7.6 Rand) included local unit costs, and two scenarios for HAART prices for WHO recommended first-line regimens: scenario 1 used current South African public-sector ART drug prices of $730 per patient-year (PPY), whereas scenario 2 was based on the anticipated public-sector price for locally manufactured drug of $181 PPY. All analyses are presented in terms of patients without AIDS and patients with AIDS.

For patients without AIDS, the mean number of inpatient days PPY was 1.08 (95% confidence interval [CI]: 0.97–1.19) for the HAART group versus 3.73 (95% CI: 3.55–3.97) for the No-ART group, and 8.71 (95% CI: 8.40–9.03) versus 4.35 (95% CI: 4.12–5.61) respectively for mean number of outpatient visits PPY. Average service provision PPY was $950 for the No-ART group versus $1,342 and $793 PPY for the HAART group for scenario 1 and 2 respectively, whereas the incremental cost per life-year gained (LYG) was $1,622 for scenario 1 and $675 for scenario 2. For patients with AIDS, mean inpatients days PPY was 2.04 (95% CI: 1.63–2.52) for the HAART versus 15.36 (95% CI: 13.97–16.85) for the No-ART group. Mean outpatient visits PPY was 7.62 (95% CI: 6.81–8.49) compared with 6.60 (95% CI: 5.69–7.62) respectively. Average service provision PPY was $3,520 for the No-ART group versus $1,513 and $964 for the HAART group for scenario 1 and 2 respectively, whereas the incremental cost per LYG was cost saving for both scenarios. In a sensitivity analysis based on the lower (25%) and upper (75%) interquartile range survival percentiles, the incremental cost per LYG ranged from $1,557 to $1,772 for the group without AIDS and from cost saving to $111 for patients with AIDS.

Conclusion

HAART is a cost-effective intervention in South Africa, and cost saving when HAART prices are further reduced. Our estimates, however, were based on direct costs, and as such the actual cost saving might have been underestimated if indirect costs were also included.

Introduction

South Africa is experiencing an HIV epidemic with enormous social and economic consequences. Recent estimates suggest that between 4.5 and 6.2 million of the 43 million South Africans are infected with HIV-1 [1]. There were 370,000 AIDS deaths during 2003 [1], and the cumulative projected AIDS mortality for 2010 is 4–7 million in absence of a highly active antiretroviral therapy (HAART) programme [2]. The largest impact of HIV on the public health sector lies in the hospital sector [3]. In the year 2000, HIV-related admissions amounted to 24% of all public hospital admissions [4] and 12.5% of the total public health budget [5]. Cost of inpatient and ambulatory health care of both private and public health-care sectors is expected to rise rapidly [5].

The cost-effectiveness of HAART, in terms of reducing HIV-related morbidity and mortality, has been documented in industrialized countries [6–12]. The introduction of combination HAART into routine clinical care in these countries has been associated with a shift from inpatient to outpatient-based hospital care [11–17]. Until recently the prevailing assumption was that the public sector of the South African health-care system was unable to afford the introduction of antiretroviral therapy (ART) in routine clinical care. However, the government of South Africa recently announced its commitment towards creating the necessary conditions for introducing ART into the public health sector [18]. In addition, the price of HAART for resource-poor countries decreased markedly since the year 2000 [19,20]. The South African Department of Health has recently awarded contracts for the supply of ART drugs to public health facilities countrywide to international pharmaceutical companies [21]. This tender is expected to reduce HAART price to $181 per patient-year (PPY).

The aim of this study was to compare use and cost of HIV-1–related service provision between patients receiving HAART and a comparison group not receiving ART, and assess the cost effectiveness of HAART.

Methods

Study Population

This study was based on the Cape Town AIDS Cohort (CTAC); a prospective cohort study which has been described previously [22,23]. In brief, patients of this cohort were accrued from the HIV clinics affiliated to the University of Cape Town, who were referred from a wide range of primary HIV health-care providers. During the study period 1st January 1995 to 31st December 2000, HAART was not available in the publicly funded South African health-care sector. All patients in this study accessed HAART through the participation in the international HAART multicentre phase III clinical trials, as approved by the Research Ethics Committee of the University of Cape Town.

For the purpose of this study, all patients who participated in the clinical trials and received at least three ART drugs—a non-nucleoside reverse transcriptase inhibitor or protease inhibitor together with two nucleoside analogues or three nucleoside analogues—were included as the treated arm of the study (HAART group). Patients were excluded from the clinical trials if they were active injecting-drug users, were diagnosed with an acute opportunistic infection at the time of recruitment, were reported to have significant laboratory abnormalities, or if they were treated with immune-modulating or systemic chemotherapeutic agents. Lactating or pregnant women were also excluded. The trial visit schedule was usually at weeks 2, 4, and 8 and then every two to three months thereafter.

Patients who did not participate in these clinical trials and never had access to ART throughout the study period (No-ART group) but received other HIV-related care were the sample from which a "comparator" group was identified for the HAART group.

At each clinic visit, all patients were routinely examined for HIV related manifestations and staged using the World Health Organization (WHO) clinical HIV staging system [24]. HIV-1 infection was diagnosed by enzyme-linked immunosorbent assay (ELISA) tests and confirmed by Western blot or a second enzyme-linked immunosorbent assay test. Viral load (which was available only for the HAART group) was determined by reverse transcriptase-polymerase chain reaction (Amplicor; Roche Molecular Systems, Branchburg, New Jersey, United States) and $CD4^+$ count, measured by flow cytometry (Beckman Coulter, Miami, Florida, United States).

Analysis

This analysis calculated the use and cost of HIV service provision and compared the clinical outcome, in terms of disease progression or life year gained (LYG) by clinical stage of HIV infection, between patients receiving HAART and a matched comparison group who did not receive ART (No-ART group). Patients were classified as either being non-AIDS (WHO stages 1, 2, or 3) or AIDS (WHO stage 4) patients.

Several strategies were employed to ensure that the two groups studied were clinically, immunologically, and socioeconomically similar and matched for the same variables used to recruit the HAART group into the clinical trials. Logistic regression models were fitted to identify factors associated with receiving HAART in this cohort using SAS GENMODE procedure with logit link function and binomial error distribution [25]. HAART patients were individually matched with randomly selected No-ART patients on the basis of variables independently associated with the likelihood of receiving HAART. The socioeconomic status of each patient was classified into "low" or "high", using a composite index developed by the Cape Metropolitan Council [26]. A subgroup logistic regression analysis was performed for the HAART group, to examine whether the likelihood of hospitalisation differed by HAART class.

To examine for residual confounding, the matched case–control data were analysed using a conditional logistic regression model, stratified by matching variables. The model was fitted using the SAS PHREG procedure with discrete logistic model. All data analyses were performed in SAS version 8.02. χ^2 was used to compare categorical variables, and the non-parametric median test was used to compare continuous non-normally distributed variables. All p-values quoted are two sided, with a p-value < 0.05 considered as significant.

Use and Cost of Services

Information on inpatient and outpatient care was obtained from the computerized hospital information systems supple-

mented by case notes. The mean number of inpatient days and outpatient visits PPY were calculated for the non-AIDS (WHO stage 1, 2, and 3) and AIDS (WHO stage 4) WHO clinical stages for both HAART and No-ART groups. A patient-year was defined as 365.25 days of follow up and methods used for calculating the mean use of services were similar to those used in other studies [12,16,17,27]. The denominator consisted of the total duration of follow up for all patients seen during the study period and numerators were calculated by summing the use of each service. Mean and 95% confidence intervals (CI) of inpatient and outpatient service use PPY by WHO stage were calculated for the two groups using the binomial distribution, and were compared between the two groups by calculating the odds ratio (OR) of the use of inpatient and outpatient services, using the No-ART group as a reference group.

The costs of hospital HIV service provision were calculated from a public health-care system perspective [28–30]. Unit costs were obtained from a detailed costing study of HIV inpatient and outpatient care conducted in the year 2000 [31], and were adjusted for inflation to financial year 2004 prices using the South African Consumer Price Index [32]. Prices were converted from South African Rand to US dollars using the average exchange rate for 2004 (US dollars = 7.6 Rand) [33]. The unit cost was $215 for an inpatient day and $33 for an outpatient visit and included costs for tests including CD4 counts, procedures, and non-ART drug costs. The non-ART drugs included all drugs other than ART dispensed to the patients during the course of care, including treatment and prophylaxis for opportunistic infections. Mean inpatient days and outpatient visits PPY were multiplied by their respective unit costs to estimate the PPY cost of service provision.

ART prices used in this study are those currently available to the public health-care sector (Ministry of Health, Provincial Administration of the Western Cape). HAART drug-price scenarios presented were (1) present public sector prices, which amounted to $730 per annum, and (2) anticipated public sector price for locally manufactured drugs, which amounted to $181 per annum, for the WHO-recommended regimen for resource-limited settings [34].

To estimate the total cost of service provision PPY for HAART patients for the two scenarios, average ART drug costs PPY were added to the average inpatient and outpatient PPY costs. In sensitivity analysis, minimum and maximum ART drug PPY costs for the two scenarios were also added to the lower and upper limit of the 95% CI: inpatient and outpatient PPY cost of care to provide a range of costs. Viral load was not measured for the No-ART group because it was not available in publicly funded institutions during the study period and, therefore, PPY cost of viral-load investigation of $79 (D. Roditti, personal communication) was only added to the annual cost of service provision for the HAART group.

Cost of LYG by WHO Stage of HIV Infection

Progression times were calculated from date of entry into non-AIDS (WHO stage 1, 2, or 3) to date of progression to AIDS (WHO stage 4) or death, and from initial diagnosis of AIDS (WHO stage 4) to death for AIDS patients. Patients not known to have progressed during follow-up were censored at either the most recent visit to the clinic or when lost to follow-up. Median progression times were estimated using the product-limit Kaplan-Meier survival method, and these were compared for the HAART and No-ART groups using log-rank test. Due to the small number of individuals who progressed during the follow-up period, median and interquartile ranges (IQR) for time to progression to AIDS or death were extrapolated from the product-limit time to failure estimates using the maximum likelihood least squares method. The progression-free times for non-AIDS and AIDS patients for each group were multiplied by the average PPY cost of service provision, and the additional life years gained of non-AIDS and AIDS groups was calculated as the incremental cost per LYG, based on the difference in the estimated median progression times of the two groups [27].

Because discounting health benefits remains controversial [35], only non-discounted estimates are presented. However, given the relatively short time in each WHO stage, it is unlikely that an analysis with a non-zero discount rate would yield qualitatively different results than those presented here.

Sensitivity Analysis

Robustness of results was assessed in a sensitivity analysis; accounting for variances associated with treatment effects and total cost of service provision. IQRs between the lower (25%) and upper (75%) progression-free times percentiles of the non-AIDS and AIDS patients were multiplied by the average and 95% CI of the cost of service provision, and the incremental cost per LYG was calculated.

Results

Study Sample

Of the 1,630 patients in the cohort, 292 patients (265 non-AIDS and 27 with AIDS) received HAART through participation in the clinical trials. The rest of the patients (n = 1,328; 1,093 non-AIDS and 235 with AIDS) did not have access to ART during the study period and comprised the population from which the No-ART comparator group for the 292 patients who received HAART was identified. Baseline CD4 count, WHO stage, age, and socioeconomic status were independently associated with the likelihood of receiving HAART (Table 1), but gender was not, and therefore this variable was not considered in further analyses. Matching was therefore based on WHO stage, CD4 count (<200, 200–350, and >350 cells/μl), age (less than the median age or equal to the median age or greater of the non-AIDS and AIDS groups respectively) and socioeconomic status (low or high socioeconomic status).

HAART drug classes were not independently associated with increased risk of hospitalisation (Table 2) and were therefore analysed as one category. The characteristics of the final study population of the 292 patients who received HAART and the 292 matched No-ART patients are described in Table 3.

The Non-AIDS Population (WHO Stage 1, 2, or 3)

The matched non-AIDS group included 265 patients both in the HAART and No-ART group. Approximately one-third of the patients in the two groups had a baseline CD4 count <200 cell/μl and (49.4%) were of low socioeconomic status. Median age at inclusion into study did not differ in the two groups; 32 y, [IQR: 28–39 y] in the HAART group versus 32 y [IQR: 28–40 y] in the No-ART group (median test $p = 0.48$).

Table 1. Univariate and Multivariate Logistic Regression Analyses of Factors Associated with the Likelihood of Receiving HAART

Characteristic	Subcategory	Univariate Analysis			Multivariable Analysis		
		OR	95% CI:	*p*-Value	OR	95% CI:	*p*-Value
CD4 count (cells/μl)	<200	1.00	0.73–1.38	0.98	1.14	0.82–1.60	0.44
	200–350	2.05	1.49–2.84	<0.001	2.14	1.54–2.99	<0.001
	>350	1			1		
WHO stage	Non-AIDS	2.10	1.39–3.21	0.001	2.24	1.43–3.49	<0.001
	AIDS	1			1		
Age	<32	0.68	0.53–0.88	0.004	0.70	0.54–0.92	0.01
	≥32	1			1		
Socioeconomic status	Low status	0.44	0.34–0.56	<0.001	0.43	0.33–0.56	<0.001
	High	1			1		
Gender status	Male	1.26	0.98–1.63	0.08	1.07	0.82–1.40	0.64
	Female	1			1		

OR, odds ratio.

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Although not matched for, gender distribution did not differ statistically in the two groups ($\chi^2 = 0.07$, $p = 0.79$; Table 3). Median progression time was significantly longer in the HAART group compared with the No-ART group at 4.1 and 3.0 y respectively (log-rank test $\chi^2 = 36.6$, $p < 0.001$; Figure 1).

Use and Cost of Services and Cost per LYG

Patients on HAART had 1.08 (95% CI: 0.97–1.19) mean inpatient days, significantly fewer than the 3.75 d (95% CI: 3.55–3.97) of the No-ART group; $\chi^2 = 147$, OR = 0.29, 95% CI: 0.23–0.36, $p < 0.001$; but had significantly more outpatient visits of 8.71 (95% CI: 8.40–9.03) compared with 4.35 (95% CI: 4.12–5.61); $\chi^2 = 145$, OR = 2.00, 95% CI: 1.78–2.25, $p < 0.0001$ (Table 4). The average PPY inpatient cost in the HAART group was significantly less than that for the No-ART group, while the average costs of outpatient visits PPY for the No-ART group were less than those for the HAART group (Table 4).

Based on the two HAART price scenarios, the average cost of service provision PPY for the HAART group ranged from a minimum of $760 to $1,377 PPY, with scenario 2 having the lowest service provision cost (Table 4). The incremental cost per LYG for median progression time was $1,622 (95% CI: 1,607–1,627) for scenario 1 and $675 (95% CI: 659–679) for scenario 2 (Table 5). When a sensitivity analysis was performed based on the IQR of the progression times, the incremental cost per LYG varied between $1,578 (95% CI: 1,557–1,581) and $1,759 (95% CI: 1,748–1,772) for the 25th and 75th percentiles respectively (Table 5).

The AIDS Population (WHO Stage 4)

The AIDS population included 27 patients in each group. The majority of patients in the two groups presented with a CD4 count <200 cell/μl (77%), and 40.74% were of low socioeconomic status. Median age did not differ in the two groups; 35 y (IQR: 32–41) in the HAART group versus 37 y (IQR: 33–50) in the No-ART group (median test $p = 0.27$). Gender distribution, with 63% and 70.4% males in the HAART and No-ART groups respectively, was not significantly different in the two groups ($\chi^2 = 0.33$, $p = 0.56$) (see Table 3). Median progression time was significantly longer in the HAART group compared with the No-ART group; at 3.1 and 1.4 y respectively (log-rank $\chi^2 = 5.28$, $p = 0.02$; Figure 2).

Use and Cost of Services and Cost per LYG

Patients on HAART had significantly fewer mean PPY inpatient days at 2.04 d (95% CI: 1.63–2.52) compared with 15.36 d (95% CI: 13.97–16.85) for the No-ART group ($\chi^2 =$ 1,019, OR = 0.13, 95 CI: 0.11–0.15, $p < 0.0001$). Mean outpatient visits PPY in the two groups did differ significantly; at 7.62 (95% CI: 6.81–8.49) for the HAART group compared with 6.60 (95% CI: 5.69–7.62) for the No-ART

Table 2. Logistic Regression Analysis of Factors Associated with Hospitalisation among the Treated Group

Variable	Subcategory	Univariate Analysis			Multivariate Analysis		
		RR	95% CI	*p*-Value	RR	95% CI	*p*-Value
CD4 count (cells/μl)	<200	2.04	1.02–4.10	0.04	1.77	0.86–3.64	0.12
	200–350	1.31	0.64–2.66	0.46	1.22	0.59–2.52	0.59
	>350	1			1		
Viral load (log_{10} copies/μl)		0.70	0.46–1.06	0.09	0.77	0.50–1.19	0.24
HAART drug class	NNRTI ($n = 154$, 52.7%)	1.23	0.71–2.14	0.13	1.16	0.67–2.04	0.59
	TNRTI ($n = 21$, 7.2%)	0.53	0.15–1.93	0.34	0.59	0.16–2.16	0.42
	PI ($n = 117$, 40.1%)	1			1		

NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RR, risk ratio; TNRTI, triple nucleoside reverse transcriptase inhibitor.

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Table 3. Baseline Demographic and Clinical Characteristics of the Matched Non-AIDS and AIDS Groups

Variable	Subcategory	Non-AIDS		AIDS	
		HAART ($n = 265$)	No-ART ($n = 27$)	HAART($n = 265$)	No-ART ($n = 27$)
		n (%)	*n* (%)	*n* (%)	*n* (%)
CD4 count	<200 cells/μl	81 (30.6)	81 (30.6)	21 (77.7)	21 (77.7)
200–350 cells/μlL	110 (41.5)	110 (41.5)	1 (3.8)	1 (3.8)	
	>350 cells/μL	74 (27.9)	74 (27.9)	5 (18.5)	5 (18.5)
Median age (IQR)		32 (28–39)	32 (28–40)	35 (32–41)	37 (33–50)
Socio-economic status	Low status	131 (49.4)	131 (49.4)	11 (40.74)	11 (40.74)
	High status	134 (50.6)	134 (50.6)	16 (59.26)	16 (59.26)
Gender	Male	142 (53.60)	145 (54.7)	17 (63)	19 (70.4)
	Female	123 (46.4)	120 (45.3)	10 (37)	8 (29.6)

IQR, interquartile range.
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group; $\chi^2 = 7.3$, OR = 1.15, 95% CI: 1.04–1.28, $p = 0.007$, though not as substantially as for the non-AIDS group (see Table 4). The average inpatient cost PPY in the HAART group was significantly less than that for the No-ART group, but the average costs of outpatient visits PPY in the groups were not significantly (see Table 4).

Based on the two HAART price scenarios, the average cost of service provision PPY for the HAART group ranged from a minimum of $850 to $1,645 PPY, with the lowest care cost observed for scenario 2 (see Table 4). For patients diagnosed with AIDS, the incremental cost per LYG for the median progression time was cost saving for both HAART price scenarios (Table 5). When a sensitivity analysis was performed based on the IQR of the progression times, the incremental cost per LYG varied between $71 (95% CI: 43–111) and cost saving for the 25th and 75th progression-free time percentiles respectively (Table 5).

Discussion

This study, employing methods used in similar studies from industrialized countries [27], provides a unique contemporaneous comparison of the use, cost, and outcome of hospital service provision for a group of HIV-infected patients in Cape Town receiving HAART compared with an immunologically, clinically, and socioeconomically similar group of patients who did not receive ART. Use of HAART was associated with decreased disease progression, AIDS, and death. The HAART group used fewer inpatient services than the No-ART group, and the magnitude of these changes did not differ by HAART regimens used in this study. The

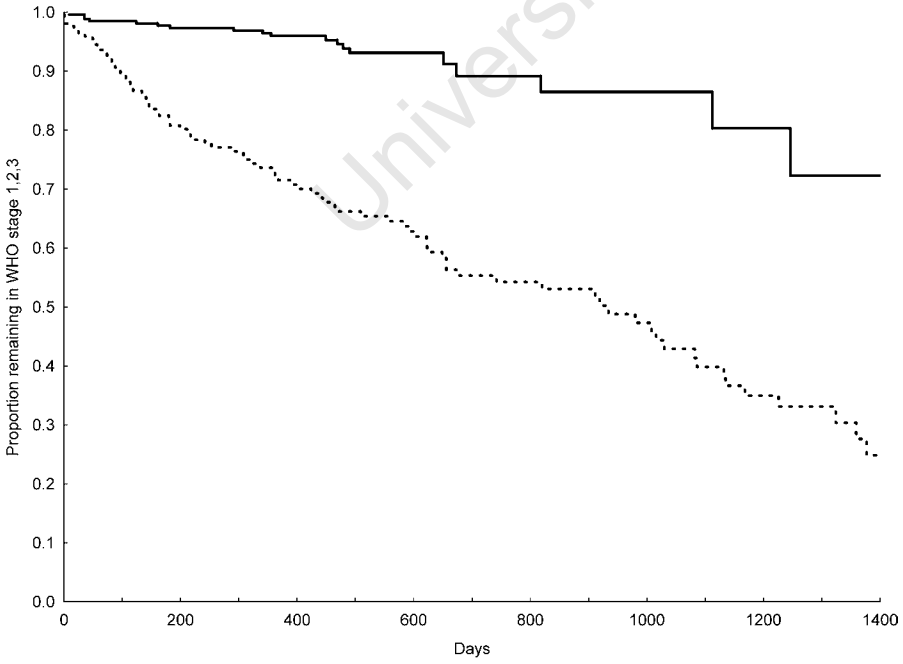

Figure 1. Progression of HIV-Infected Individuals from Non-AIDS Stages (WHO Stage 1, 2, or 3) for Patients on HAART and Not on ART
The solid line indicates patients on HAART, and the dotted line indicates patients not on ART.
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Table 4. Mean Number of Inpatient Days, Outpatient Visits, and Associated Cost[a] PPY

Variable	Non-AIDS		AIDS	
	HAART	No-ART	HAART	No-ART
Mean number of inpatient days PPY (95% CI)	1.08 (0.97–1.19)	3.75 (3.55–3.97)	2.04 (1.63–2.52)	15.36 (13.97–16.85)
	OR[a] = 0.29 (95% CI: 0.23–0.36)		OR = 0.13 (95% CI: 0.11–0.15)	
Average inpatient days cost (95% CI)	$232 (209–256)	$806 (763–854)	$439 (351–542)	$3,302 (3,004–3,623)
Mean number of outpatient visits PPY (95% CI)	8.71 (8.40–9.03)	4.35 (4.12–5.61)	7.62 (6.81–8.49)	6.60 (5.69–7.62)
	OR = 2.00 (95% CI: 1.78–2.25)	OR = 1.15 (95% CI: 1.04–1.28)		
Average outpatient visits cost (95% CI)	$287 (277–298)	$144 (136–185)	$251 (225–280)	$218 (188–252)
Average total cost PPY (95% CI)		$950 (899–1,039)		$3,520 (3,192–3,875)
Scenario 1 (current public sector price = $730)	$1,342 (1,309–1,377)		$1,513 (1,399–1,645)	
Scenario 2 (anticipated tender price = 181)	$793 (760–828)		$964 (850–1,096)	

[a]Odds ratio (OR) uses the No-ART group as baseline risk.

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reduction in use of inpatient services, which has been observed in similar studies in industrialized countries [10–15], was most likely due to a reduction in morbidity and mortality [6,12]. The use of services increased for both groups with increased severity of HIV infection, resulting in an increased cost of service provision. The increased use of inpatient services for patients with AIDS is most likely related to AIDS-related events or their terminal phase of their illness [36–41]. In Zimbabwe, medical insurance claims of privately insured HIV-infected patients in the last few months of their lives were 700% higher than that of uninfected patients in the same age group [42].

To date, very few cost-effectiveness studies have been performed on HAART in a South African setting [43]. The incremental cost per LYG ranged from being cost saving to $1,772. The cut-off point for what constitutes a cost-effective intervention per outcome measure varies from society to another. For instance, the cost-effective cut-off point in the United States is currently considered to be $50,000 per outcome measure and £30,000 in the United Kingdom [30]. To date such a consensus on what would constitute a realistic threshold for South Africa has not yet emerged, but a cut-off of twice the per capita gross domestic product (GDP) has been suggested as a reasonable cut-off point for developing countries [44]. For the year 2004, the per capita gross domestic product in South Africa was $3,480, and therefore this threshold would amount to $6,960 [45]. The cost per LYG of two HAART cost scenarios for the non-AIDS and AIDS patients showed that introducing HAART in this hospital setting would be a very cost-effective intervention. However, it is clear that the cost-effectiveness ratios were very sensitive to the price of HAART. If prices of the awarded tender could be achieved, the introduction of HAART will be a very cost-effective intervention in Cape Town and probably in similar settings in sub-Saharan Africa, because HIV accounts for between 40% and 70% of the public sector inpatient service provision in the region [3,36–40].

Concern has been expressed that increased access to

Table 5. Incremental Cost-Effectiveness Ratio (US$) for Current ART Rollout Prices (US$730 Per Annum—Scenario 1) and Anticipated Tender Prices (US$181 Per Annum—Scenario 2), Comparing HAART and No-ART Groups for Non-AIDS and AIDS Groups at 25th, 50th (Median). and 75th Progression-Free Times Percentiles

Survival Quartile	Group	Survival Time (d)	ICER (95% CI)	
			Scenario 1[a]	Scenario 2[b]
25%	Non-AIDS	HAART (1391)	$1,578 (1,557–1,581)	$698 (676–701)
		No-ART (523)		
	AIDS	HAART (739)	$71 (43–111)	Cost-saving
		No-ART (309)		
Median 50%	Non-AIDS	HAART (2,641)	$1,622 (1,607–1,627)	$675 (659–679)
		No-ART (1,111)		
AIDS	HAART (1,120)	Cost-saving	Cost-saving	
	No-ART (510)			
75%	Non-AIDS	HAART (3,891)	$1,759 (1,748–1,772)	$608 (597–621)
	No-ART (2,035)			
AIDS	HAART (1,561)	Cost-saving	Cost-saving	
	No-ART (980)			

[a]Current rollout prices.
[b]Anticipated tender prices.
ICER, incremental cost-effectiveness ratio.

DOI: 10.1371/journal.pmed.0030004.g005

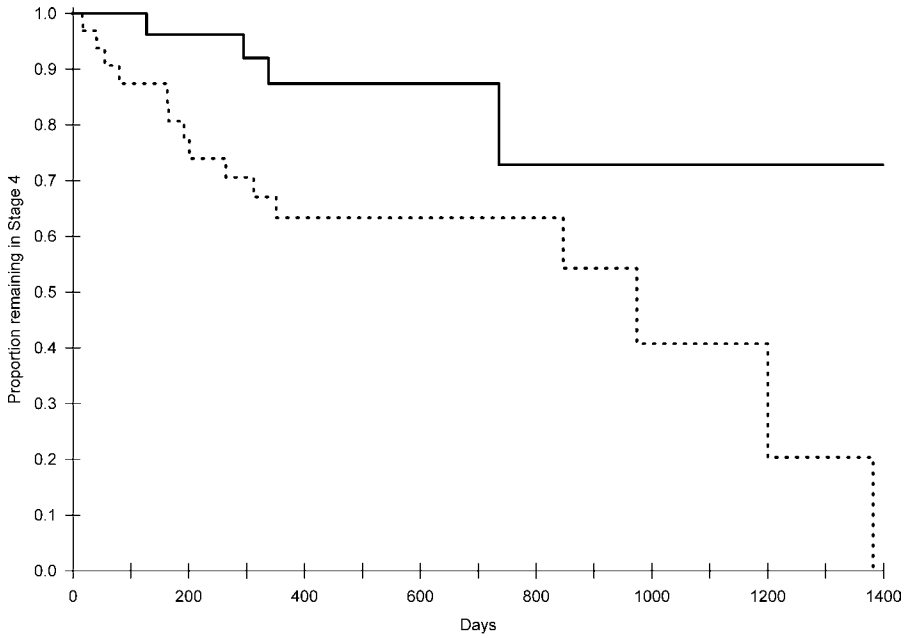

Figure 2. Progression of HIV-Infected Individuals from WHO Stage 4 for Patients on HAART and Not on ART
The solid line indicates patients on HAART, and the dotted line indicates patients not on ART.
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HAART in sub-Saharan Africa will result in the widespread viral resistance due to poor adherence [46]. Studies performed in a number of sub-Saharan African countries, however, have shown that the proportion of individuals maintaining viral suppression is comparable to that reported from developed countries [47–49].

This study did have a number of limitations. Because HAART was not used in routine clinical practice, we had to compare a group of patients enrolled in clinical trials with a control group that was not part of the trials. Individuals who take part in clinical trials have to fulfil certain entry criteria, as well as to conform to well-defined protocols and scheduled attendances. It is therefore difficult to exclude the possibility that a selection bias might have resulted from the inclusion/exclusion criteria of these clinical trials. However, the No-ART control group was selected on the basis of clinical, socioeconomic, and immunologic characteristics similar to those individuals recruited into the HAART trials conducted in this study. The frequency of inpatient and outpatient services utilization of the HAART and No-ART patients in this study is similar to that reported by UK and Canadian observational studies [12,27]. However, in this study, the sample is relatively small for the AIDS group.

This study was focused on hospital services provided at the level of a teaching hospital. Therefore the costs incurred through primary, community, or secondary hospital care were not included, but this reflected the configuration of services available to the majority of HIV-infected people in Cape Town at the time of the study. Similarly, the costs included were direct costs only and did not incorporate the indirect or intangible costs, such as loss of productivity or quality of life associated with this illness, because currently no such data exist in South Africa. Some studies from the United Kingdom have demonstrated that from a public sector perspective, indirect costs can comprise between 58% and 124% of direct treatment costs for HAART or between 45% and 102% from a societal perspective [30]. If these costs were all included, it is likely that the cost-effectiveness ratio would even be more favourable. Our estimates did not incorporate the costs of providing the infrastructure required to support appropriate HAART provision in rollout programmes. However the rollout programmes were designed to start from settings where infrastructure currently exists, which would predominantly be urban. Recent reports estimated that if the public sector included HAART as part of a package of HIV treatment and care in the year 2003, the cost would be 1.2% of the South African GNP, which is unlikely to push health-care expenditure beyond prudent levels [50,51].

The recent commitment towards scale-up of HAART in South Africa as part of HIV treatment and care has been an important and positive development. The urgent need to introduce HAART as part of routine HIV treatment and care was recently re-iterated in a World Bank report, which indicated that if this is not done soon, failure to do so would have devastating effects on this and future generations of South Africans [52].

Although the primary rationale for wider access to HAART is humanitarian, a national HAART programme targeting patients with symptomatic HIV disease, using low-cost HAART prices would also significantly decrease hospital services utilization by HIV-infected patients, resulting in either health expenditure saving by cost deferral or freeing substantial resources for health care of non-HIV patients.

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Patient Summary

Background The number of cases of AIDS continues to increase worldwide; the disease is a major threat to humanity, with Africa facing the very worst problems. In South Africa alone there were 370,000 AIDS deaths in 2003. AIDS is caused by a type of retrovirus—the human immunodeficiency virus (HIV). Highly active antiretroviral treatment (HAART) is a treatment that uses a combination of three or more antiretroviral drugs that attack different parts of the virus. HAART is expensive, making it difficult for poor countries to provide treatment for all those who need it. Prices are falling, however, and South Africa is one country where efforts are now being made to improve access to treatment.

Why Was This Study Done? The cost-effectiveness of HAART has been studied in developed countries, but developing countries also need to know how much it is going to cost their health services if they introduce HAART, and whether there will be financial savings because of switching to a more effective treatment.

What Did the Researchers Do and Find? During the study period (January 1995 to 31st December 2000), HAART was not available in the publicly funded South African health-care sector. The study, funded by the drug manufacturer Bristol-Myers Squibb, took place in HIV clinics affiliated to the University of Cape Town. The researchers compared the cost of services for 292 patients who were given HAART with the costs for a comparison group (with the same number of patients) who were not given any antiretroviral drugs. Twenty-seven patients in each group had AIDS; the others were HIV-infected but did not have AIDS. The researchers calculated costs per patient year (PPY) and per life-year gained (LYG), i.e., the total cost divided by the number of extra years the treated patients lived. Calculations were done separately for patients with AIDS and those without AIDS. Patients on HAART required fewer hospital admissions. Depending on how long the patient survived and the price of antiretrovirals drug it cost less to treat the HAART patients with AIDS. For this group, the cost saving ranged from $219 to $2,116 (in U.S. dollars). For patients without AIDS, the cost of treatment (ranging from $597 to $1,772) was, by the South African standard of cost of living, affordable. However, it is expected that South Africa will soon be able to manufacture antiretroviral drugs locally and more cheaply. This would increase the amount saved by introducing HAART.

What Does This Mean? HAART seems to be a more cost-effective way for South African hospitals to treat HIV infection than by simply waiting for patients to come to hospital and then dealing with their symptoms. However, it should be noted that, when a person is infected with HIV and becomes ill or dies from AIDS, it is not only hospitals that face costs. The patient, their family, and the country suffer financially. Effective treatment might also lower these "indirect" costs, but this was not an issue examined in this research.

Where Can I Find More Information Online? For a comprehensive source of information on HIV/AIDS:
http://www.thebody.com
The site also includes a useful section on HAART:
http://www.thebody.com/Forums/AIDS/Infections/Archive/NewMedications/Q12178.html.
For information about the global AIDS situation and the position in different countries:
http://www.unaids.int.

Antiretroviral therapy in a community clinic — early lessons from a pilot project

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Objectives. To report on operational and clinical problems encountered during the first 6 months of a community-based antiretroviral therapy (ART) programme.

Methods. ART was implemented in a primary care setting utilising an easily replicable service-delivery model based on a medical officer and nurse. Therapeutic counsellors, themselves HIV-infected, provided counselling and adherence support. Drug and monitoring costs were charitably funded and provincial health authorities supplied the medical infrastructure. The HIV Research Unit, University of Cape Town, supplied training and additional clinical support. Local HIV primary care clinics provided patient referrals. Standardised ART regimens were used with strict entry criteria (AIDS or CD4 count < 200 cells / µl).

Results. Demand for the service was high. Referred patients had advanced disease (AIDS 57%, median CD4 count 96 / µl) and high pre-treatment mortality (83 / 100 person-years). Mycobacterial disease was a major contributor to this mortality (40%). Scheduled clinic visit hours were six times higher during recruitment than maintenance. Attributable costs were: drugs 61%, staff 27%, viral load and CD4 cell counts 10% and safety monitoring 2%. Viral load after 16 weeks of therapy was < 400 copies / ml in the first 16 patients.

Conclusions. ART can be successfully implemented within a primary care setting. Drug purchases and staff salaries drive programme costing. The service model is capable of managing 250 - 300 patients on chronic ART, but staffing needs to be increased during recruitment. Attention must be given to the diagnosis of tuberculosis during screening and early ART. Incorporating therapeutic counsellors into the programme increased community involvement and utilised a valuable and previously untapped resource.

S Afr Med J 2003; **93:** 458-462.

Combination antiretroviral therapy (ART) has substantially improved the prognosis of HIV-infected individuals, resulting in a precipitous drop in AIDS-related mortality in affluent countries.[1-3] In contrast, in the most heavily burdened developing countries of the world, the response to the epidemic has focused on prevention. Prevention strategies have not been successful and the combination of large numbers of HIV-infected individuals compounded by unequal access to medical resources, has resulted in an ever-widening life expectancy gap between wealthy and developing countries.[4] In response to this crisis, the World Health Organisation (WHO) has called for expanded access to ART in resource-poor countries.[5] ART programmes have been successfully incorporated into highly divergent medical environments. Brazil, a middle-income developing country, has incorporated ART into its public health system[6] and a successful ART programme has been implemented in rural Haiti, the poorest country in the Western hemisphere.[7]

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The Usapho Lwethu *Project Team, from left to right: Dr Kwezi Matoti, Poppy, Nomaroma, Mtateleli, Nobafundi, Sr Felicity Cope, Noluvo and Nonsikelelo.*

In South Africa, AIDS is now the major cause of death among young adults.[8] While ART is available to thousands of South Africans in the private medical sector,[9] there is little or no ART access in the public sector, despite demands for such therapy from the HIV-infected community.[10] It has been argued that 'where HIV is the leading cause of death, a basic minimum package that does not include antiretrovirals is not worthy of the name'.[7]

In this paper we report on the initial medical, ethical and logistic challenges of initiating a community-based ART pilot project in a district where there is an existing network of HIV primary health care (PHC) clinics. The project was called *Usapho Lwethu* (My Family), since treatment was offered to individuals and their partners and children wherever appropriate. An easily replicable service delivery model was chosen based on a team consisting of a medical officer, nurse and therapeutic counsellors. In order to minimise operational complexity, standardised ART regimens were used[11,12] which complied with international[5] and national treatment guidelines.[13] To facilitate expansion of this or similar service delivery models for ART elsewhere in South Africa, there is urgent need to report and share knowledge of the operational challenges that need to be overcome.[10]

Methods

The ART clinic was situated in the Guguletu Community Health Centre in the Nyanga district of Cape Town, which has a population of 325 436 (projected figure from 1996 census data). There are 10 primary care HIV clinics within the district, which served as the patient referral base. Each clinic had trained staff able to provide voluntary testing and counselling services together with prophylaxis and outpatient management of common HIV opportunistic infections. Patients requiring inpatient care were referred to a local 200-bed secondary hospital.

Funding for antiretroviral medication, viral load measurements and CD4 cell counts, sufficient for 150 patients, was provided by UK-based charities. The local Community Health Service Organisation and AIDS Directorate of the Western Cape provided clinic space and medical support services, together with dedicated clinic staff, consisting of a medical officer and nurse. Training, clinical support, protocol development and programme evaluation were provided by the HIV Research Unit of the University of Cape Town.

In August 2002, medical staff of the referring PHC clinics were invited to workshops, where eligibility criteria and patient referral mechanisms were discussed. Referring doctors were requested to select suitable drug-naïve candidates from their regular clinic attendees and to fax contact details and a medical summary to the programme nurse. The medical criteria for ART eligibility were either a prior AIDS diagnosis or a CD4 cell count less than 200 cells / µl.[5]

Branded medications registered with the Medicines Control Council of South Africa were used in standard doses. ART consisted of two schedules, an initial non-nucleoside reverse transcriptase (NNRTI)-based regimen of stavudine (d4T), lamivudine (3TC) and efavirenz (nevirapine was substituted for efavirenz in women of child-bearing potential), with a second protease inhibitor (PI)-based regimen of zidovudine (AZT), didanosine (ddI) and Kaletra. Medication was sourced from a single pharmaceutical supplier with a rapid delivery turnaround time, to minimise on-site drug stocks. Drugs were packaged for each named patient and supplied in 4 x 30 day packs and stored in a secure locked section of the main clinic pharmacy.

During September 2002, the first patients were recruited into the programme, with a schedule of visits as follows: –4 weeks, –2 weeks, treatment initiation, +4 weeks, +8 weeks, +16 weeks and then at 16-week intervals. The early schedule of visits was truncated for women in the latter stages of pregnancy, to maximise therapeutic benefit to mother and baby. At the screening visit (week –4), potential candidates were allocated a therapeutic counsellor, whose responsibility was to provide ongoing counselling support, to reinforce the need for high levels of adherence, to maintain communication between clients and the clinic staff and to visit clients in their homes. The therapeutic counsellors were HIV-infected individuals, many on ART themselves, who had been trained in drug adherence and ART toxicity recognition by a non-governmental organisation, *Sizophila*. The therapeutic counsellor / patient ratio was 1:20. At this initial visit, a treatment readiness assessment questionnaire was completed and 4 weeks of co-trimoxazole were dispensed, with pill counts performed after 14 and 28 days to assess adherence. All patients had a checklist completed, which screened for symptoms and signs of tuberculosis (TB) infection. At the week –2 visit, blood was taken for quantitative viral load and CD4 cell count. These tests were repeated at 16-week intervals thereafter. Drug safety monitoring of liver function test (LFT) and full blood count (FBC) were also performed at weeks –2, +8 and 16-weekly thereafter, with an additional LFT performed at week +2 in those receiving nevirapine to screen for potential hepatotoxicity. The final decision to commence or defer initiation of ART was made at a combined meeting of medical and therapeutic counsellors during the third week (week –1), when information on clinical status, blood tests, treatment-readiness and adherence data were available. Following this meeting, drugs were ordered for those commencing ART. The cohort pre-ART period was defined as the cumulative number of days between the screening visit and the date of either commencement of ART or permanent deferral. The ART exposure period was defined as the cumulative number of days from ART commencement to the censoring date of

28 February 2003. Resource utilisation data including scheduled and non-scheduled visits and staff allocated time were prospectively collected in a clinic register. Clinical, laboratory and adherence data were kept in an electronic database.

Results

The number of eligible patients referred and their subsequent status within the programme are outlined in Fig. 1. The mean age of those screened was 34 years (range 7 - 55 years); 78% were female. The population had advanced HIV infection, with 95% having symptomatic disease (WHO clinical stages 3 and 4) and 57% having AIDS (WHO clinical stage 4). The median CD4 count was 96 cells / µl (range 3 - 452 cells / µl), and the median viral load was 4.91 logs (range 2.611 ≥ 500.000).

Eight patients were permanently deferred; 4 because of failure or inability to attend clinic visits, 1 because of advanced debilitating HIV disease and multidrug-resistant pulmonary tuberculosis, 2 because of asymptomatic HIV and CD4 cell counts above entry criteria at screening, and 1 because he denied being HIV-infected. One patient was withdrawn after therapy commenced because of failure to attend clinic visits. Viral load data at 16 weeks were available for the first 16 patients, all of which were < 400 copies / ml. The first child born to a mother on the programme was HIV PCR-negative at 3 months.

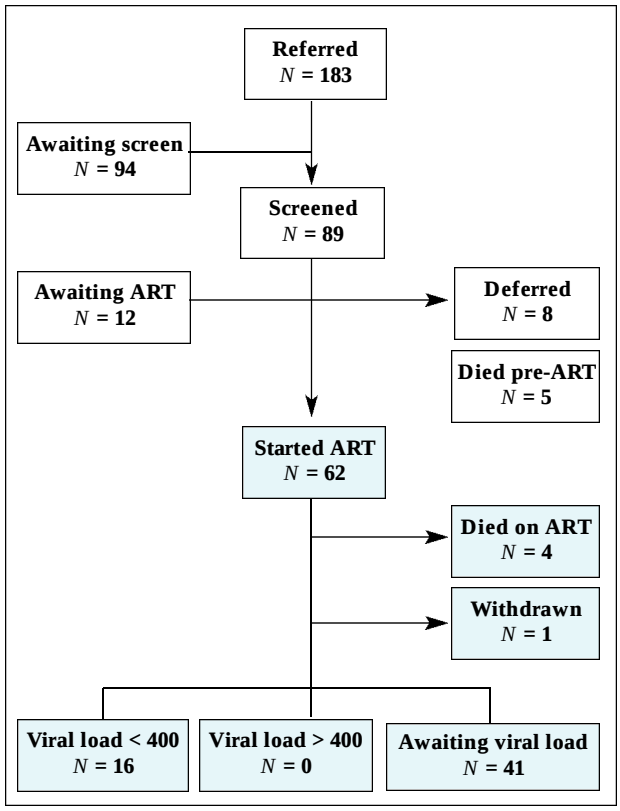

Fig. 1. Numbers of patients referred, screened and started on the antiretroviral therapy (ART) programme, together with their status within the programme and clinical and virological outcomes at week 16. Events occurring on ART are shown in the shaded boxes.

Five deaths occurred between screening and the commencement of ART or treatment deferral. The total pre-ART period for the cohort was 2 186 days, which translated to a pre-treatment crude mortality rate of 83 / 100 patient years. Two of the five patients had active *Mycobacterium tuberculosis* infection. Four deaths occurred on ART, none of which was directly related to ART. The period of ART exposure up to the censoring date totalled 4 271 days.

The proportional costs of drugs, staffing, quantitative viral load with CD4 cell counts and laboratory safety monitoring are shown in Fig. 2. Programme costs were dominated by drug procurement and personnel costs. Of the personnel costs, the medical officer contributed 39.3%, the nurse 16%, *Sizophila* counsellors 15.7% and specialist medical support and training 29%.

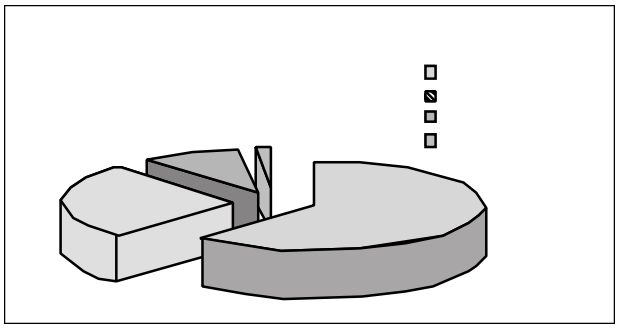

Fig. 2. The first 6 months' financial costs of the programme introducing ART to an HIV primary care service. The attributable proportion of costs for the following components are shown: drug acquisition; staffing — medical, nursing and counsellors; monitoring of CD4 and viral load; and laboratory safety monitoring.

Patient referrals reached 150 after 8 weeks of the programme. Initiation of the 150 patients onto ART was scheduled over 9 months, with a mean of 15 patients starting therapy per month. In the first 6 months there were 421 scheduled and 20 unscheduled visits. Duration of scheduled visits varied between 15 minutes for a week-8 visit and 45 minutes for a treatment-initiation visit. The number of scheduled clinic hours per week was not evenly distributed and the projected weekly scheduled visit hours required for the programme are shown in Fig. 3.

Discussion

This ART pilot project is a unique collaboration between funding organisations from a developed country, local and

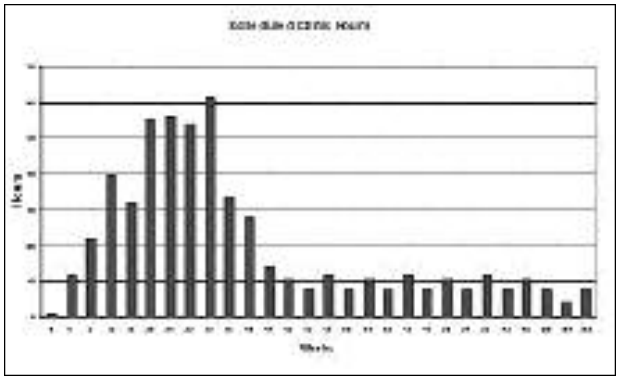

Fig. 3. Number of hours of scheduled visits per week required for 150 patients initiating HAART over a 9-month period. There is an initial staffing requirement, which peaks at approximately six times the long-term staffing needs.

regional public sector health authorities and a local academic institution. The programme was designed to identify the attributable resources needed when adding an ART programme to an existing primary care HIV service using an easily reproducible service model.

Public debate around treatment access, together with the large numbers of HIV-infected individuals with advanced disease, has created a backlog of demand for access to ART. The programme was initially limited by funding sources to treatment of 150 individuals. Recruitment to the programme was completed by 8 weeks, confirming a high demand for ART in our patient population. Many patients are desperate to access therapy and perceive that they may not survive a delay of several weeks. The clinic nurse highlighted this therapeutic urgency when she reported her distress at 'watching patients' faces, informed of the waiting time before they access antiretroviral therapy'.

Initiation of ART was staggered over 9 months, which still resulted in staffing time requirements peaking at approximately six times the level required for subsequent long-term management of these patients. The high clinic time requirement resulted from the combined effects of the recruitment rate, increased frequency of protocol-required visits and increased time per visits during the early weeks of ART initiation. The team of medical officer, nurse and therapeutic counsellors allocated to the clinic would be sufficient to supply 20 hours per week of scheduled clinic visits, enough for a daily clinic of 4 hours' duration. This scheduled clinic time could service 250 - 300 patients on stable ART; however, it is insufficient for the recruitment and initiation phase of the ART programme. In any new programme this increased staffing requirement will coincide with the need for staff training in ART. Our training personnel were able to provide the extra staffing capacity during the recruitment phase. There will be a need for programmes to allocate increased logistical support to ART clinics during the initial 'set-up' phase.

Patients on the programme had a functional status sufficient to be able to attend an outpatient clinic; however, the pre-treatment crude mortality rate was 83/100 patient years. The on-treatment mortality showed significant decline; the mortality rate has not been presented at this stage because the drug exposure period is subject to a major confounder of 'right censoring' and these data will be reported later. TB was a significant contributor to mortality and the exclusion of active TB presents a particular challenge to those initiating ART in high TB-prevalence populations such as South Africa.[14,15]

Present treatment guidelines[5,13] give an upper threshold of 200 CD4 cells/µl for initiating therapy, but there is no lower limit. This introduces the dilemma of whether patients are ever too sick to join a programme. While the sickest patients have much to gain, they utilise programme resources disproportionately and a high death rate can impact negatively on community perceptions of the programme and affect staff morale adversely. The non-medical therapeutic counsellors required specific counselling support around this issue. In an analysis of 12 574 individuals on ART, the CD4 count and clinical stage at the time of initiation of treatment were the dominant prognostic factors.[16] In a cohort with very advanced HIV disease and low CD4 counts such as ours, between 11% and 15% would be predicted to develop further AIDS-related complications during the first year of therapy, with the majority of these events occurring within the first 6 months.[16] Despite this high predicted complication rate, European and North American experience indicates that only 1.5 - 3.1% of advanced patients with 50 - 100 CD4 cells/µl die in the first year of ART.[16] These data support initiation of therapy at low CD4 counts, which can still result in a reasonable prognosis if the initial complications around the time of starting therapy can be managed. Staff of the clinic and referral hospital therefore need a high level of expertise in the recognition and management of medical complications occurring during early ART, particularly if very ill patients are to be entered into a programme.

Drug procurement accounted for 61% of the attributable cost of adding ART to the existing primary care HIV management programme. Although retail costs of ART have declined in South Africa in recent years, they remain a major obstacle to wider implementation in the public sector. In the next few months the programme is expected to access UNAIDS preferential pricing which will reduce the cost of the initial regimen to approximately 50% of current retail pricing. Availability of generic formulations could reduce the cost of our initial schedule by an additional 50%, to approximately R350 per month.[12,17] Medical specialist support contributed 29% of initial staffing costs; however, this component will also decrease as training requirements decline and an increasing

proportion of patients are stabilised on ART. Viral load and CD4 cell measurement contributed 10% of total attributable expenditure. Until recently these high-technology assays were considered to be too expensive for 'low-cost programmes' and the use of low-technology substitutes such as total lymphocyte counts was proposed.[5] The recent marked cost reduction of these assays in South Africa makes low-technology substitution less attractive.

The preliminary viral response data are very encouraging and demonstrate that an ART programme can be initiated successfully within an existing primary care facility. The ART delivery model chosen was well suited for those districts with an existing HIV primary care infrastructure. Expansion to other sites will be dependent not only on funding for antiretrovirals, but also on the allocation of sufficient resources for the development of a training and support infrastructure, particularly during the early recruitment phase of the programme. There are significant ethical issues concerning the selection of patients into an ART programme where resources are very limited. While there is a need for clear, transparent and strictly maintained criteria for ART access,[10] both the inclusion and exclusion of very sick patients was very challenging and stressful for the clinic team. Therapeutic counsellors gave valuable input to the programme from persons in the local community living with HIV and AIDS. The incorporation of HIV-infected individuals as 'treatment buddies' and to give counselling support meant that a previously untapped resource of expertise and talent was utilised.

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Initiating highly active antiretroviral therapy in sub-Saharan Africa: an assessment of the revised World Health Organization scaling-up guidelines

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Objectives: To assess the utility of the 2003 revised World Health Organization (WHO) criteria [initiating highly active antiretroviral therapy (HAART) in stage IV, in stage III plus CD4 cell count $< 350 \times 10^6$ cells/l, or in stage I or II plus CD4 cell count $< 200 \times 10^6$ cells/l] relative to other scenarios of HAART initiation.

Methods: Progression to AIDS and death in 292 patients taking HAART and 974 not taking HAART in a South African institution in 1992–2001, stratifying patients by baseline CD4 cell count and WHO stage.

Results: HAART was associated with decreased AIDS [adjusted rate ratio [ARR], 0.16; 95% confidence interval (CI), 0.08–0.31) and death (ARR, 0.10; 95% CI, 0.06–0.18). Benefit of HAART was significant across all WHO stages plus CD4 cell counts. The greatest number of deaths averted was in stages IV [74.0/100 patient-years (PY); 95% CI, 50.2–84.5) and III (32.8/100 PY; 95% CI, 22.4–40.9). AIDS cases averted in stage III (22.0/100 PY; 95% CI, 6.1–26.9) were higher than in stage I and II with CD4 cell count $< 200 \times 10^6$ cells/l (8.9/100 PY 95% CI, 5.6–13.3). Treatment initiation for symptomatic disease resulted in greater benefits than using any CD4 cell thresholds. Application of WHO criteria increased the treatment-eligible proportion from 44.5% to 56.7% ($P < 0.05$) but did not prevent more death ($P > 0.05$) than treating symptomatic disease.

Conclusion: Implementation of the revised WHO guidelines in sub-Saharan Africa may result in a significantly increased number of individuals eligible for treatment but would not be as effective a strategy for preventing death as treating symptomatic disease.

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Keywords: HAART, AIDS, mortality, sub-Saharan Africa, WHO guidelines

Introduction

Antiretroviral therapy has significantly improved the prognosis of individuals with HIV-1 disease in the developed world [1–9]. Therapy has evolved from treatment with a single nucleoside to highly active antiretroviral therapy (HAART), which is presently the standard of care. The potency of HAART has been shown by comparison with dual nucleoside therapy in the ACTG 320 study [10] and other trials [11–13], together with a temporal association with sustained decreases in AIDS-related morbidity and mortality [1–9]. However, placebo-controlled studies of HAART have not been conducted for underlying ethical considerations.

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The initial 'hit early hit hard' strategy has been moderated following increased recognition of long-term adverse events associated with HAART to one of defering treatment to a later stage of HIV immune suppression [14]. In recent years, there has been some convergence of the European and North American treatment guidelines, but precise CD4 cell count and viral load thresholds for therapy initiation still vary and undergo regular revision [15–17]. Benefits of HAART demonstrated in the developed world may differ for populations in sub-Saharan Africa because of the varying spectrum of opportunistic infections and the level of immune suppression at which they occur [18]. However, determination of the accurate threshold for HAART initiation is of particular economic relevance to large-scale programmes as it defines the proportion of the HIV-infected population eligible for treatment.

The World Health Organization (WHO) has set a target of treating three million individuals in the developing world with HAART by 2005 [19]. The 2002 WHO guidelines for scaling up antiretroviral therapy in resource-limited settings recommended initiation of HAART for those with AIDS and WHO stages I, II or III with CD4 cell counts $< 200 \times 10^6$ cells/l [20]. The 2003 guidelines revision recommended extending treatment to those with WHO stage III disease with CD4 cell counts of $200–350 \times 10^6$ cells/l [21]. In sub-Saharan Africa, frequent manifestations of WHO stage III disease include pulmonary tuberculosis, oral hairy leukoplakia and mucosal candidiasis [22–27], which have been independently associated with progressive immune suppression [24–33] and occur in more than 30% of African patients at a CD4 cell count $> 200 \times 10^6$ cells/l [22,23,27]. In addition, laboratory capacity may not be routinely available in primary health-care sites in resource-limited settings. Therefore, it would be relevant to explore low-cost strategies based on clinical parameters to identify and refer eligible patients to HAART programmes.

The present study is a comparative stratified analysis to assess the utility of the revised WHO criteria relative to other possible scenarios of HAART initiation, comparing the rates of disease progression to AIDS and death in a group of indigent patients accessing HAART and a group attending the same institution in Cape Town, South Africa who did not have access to HAART.

Methods

Setting and patients

The setting and methods of enrolment into the prospective Cape Town AIDS Cohort (CTAC) have been described previously [34]. In brief, the study site was a specialized HIV clinic at New Somerset Hospital, a major public health-care provider for HIV-infected patients in Cape Town. Antiretroviral therapy was not provided by the public health services during the study period. Patients presenting to the HIV clinic between 1995 and 2001 had limited access to HAART by participation in clinical trials. Informed consent was obtained from all participants, and studies were approved by the University of Cape Town Research Ethics Committee. Study inclusion criteria were age > 16 years but specific entry CD4 cell counts and viral loads differed between studies [34]. Exclusion criteria included clinically significant laboratory abnormalities, acute opportunistic disease, substance abuse, pregnancy, lactation or recent use of systemic chemotherapeutic agents or immune-modulating agents. All patients treated with HAART received at least three antiretroviral drugs: a non-nucleoside reverse transcriptase inhibitor or protease inhibitor together with two nucleoside analogues or three nucleoside analogues. Patients were followed every 2–3 months or more frequently if deemed clinically necessary. A prospective log of medication dispensed at each attendance was maintained. Adherence was monitored by clinic-based tablet refills. Viral load was determined by reverse transcriptase polymerase chain reaction (Amplicor; Roche Molecular Systems, Branchburg, New Jersey, USA).

A comparison group (no-HAART group) who did not access HAART was identified. This group included all patients who presented to the HIV clinic between 1992 and 2000 and did not participate in the clinical trials or access HAART privately. Patients in the no-HAART cohort were followed-up approximately 3–6 monthly or when clinically indicated. Viral load was not available in publicly funded health-care facilities and, therefore, was not measured in this group.

For both groups, HIV-1 infection was confirmed by enzyme-linked immunosorbent assay or Western blot on two different blood specimens. CD4 cell counts were measured by flow cytometry (Beckman Coulter, Miami, Florida, USA). At each attendance, clinical information was recorded, and HIV disease staged using the WHO staging criteria [35]. Socioeconomic status of each patient was defined using the Cape Metropolitan Council suburbs composite index, which has been described previously [34].

Statistical analysis

The primary end-points in this study were AIDS and death. Time to these events was defined, respectively, as time from the initial clinic visit (or from starting treatment for the HAART group) to the date of first AIDS-defining illness, and/or date of death, date of last known clinic visit or end of study period. Death events were identified from patients' records and hospital or

municipality death registry. Patients switching from the no-HAART group to the HAART group contributed survival time to the no-HAART group from their initial clinic visit till the date of initiating HAART, and subsequently to the HAART group until the date of onset of AIDS (or death), the last follow-up visit or study end-point.

The Kaplan–Meier technique and the generalized log-rank test were used to plot and compare AIDS-free and death-free survival probabilities curves of the two groups. Because the WHO guidelines utilize a combination of clinical stage plus CD4 cell count for treatment initiation [21], analyses were further stratified by baseline CD4 cell count, WHO stage and a combination of WHO stage III with CD4 cell count < 200, 200–350 or $> 350 \times 10^6$ cells/l.

Cumulative incidence rate of AIDS and death was defined as the number of events occurring in each group per 100 patient-years (PY) of follow-up. Patients presenting with prior AIDS-defining illness were excluded from the calculation of incidence rate of AIDS. The analyses were further stratified by baseline CD4 cell count and WHO stage. In addition, relative hazards from Cox multivariate proportional hazards regression models, described below, were used to calculate number of AIDS or death events averted by HAART. Binomial distribution was used to calculate the 95% confidence (CI) for incidence rates and number of AIDS and death averted. The chi-square test was used to compare differences in proportions. All calculated *P* values were two sided.

Cox proportional hazard regression analysis was used to identify variables associated with the likelihood of AIDS or death using SAS software version 8.2 (SAS, Cary, North Carolina, USA). A number of baseline prognostic variables were examined in analysis, including CD4 cell count, WHO stage, prophylactic cotrimoxazole, age, socioeconomic status, gender, and year of initial care. The assumption of proportional hazards was examined by plotting the log [−log (survival function)] estimates against log time. Interaction terms were not included in the final models as they were not significant and did not impact on the fit of the models.

The relative utility of the 2003 revised WHO treatment guidelines was assessed by comparing the projected number of patients eligible for HAART and the resulting number of deaths averted applying five different scenarios of initiating HAART in the 974 no-HAART group: scenario I the revised WHO treatment guidelines (treating patients with stage IV irrespective of CD4 cell count enumeration, with stage III plus CD4 cell count $< 350 \times 10^6$ cells/l, with stage I or II plus CD4 cell count $< 200 \times 10^6$ cells/l); scenario II the previous WHO treatment guidelines (treating patients with stage IV irrespective of CD4 cell count enumeration, with WHO stages I–III with CD4 cell count cell count $< 200 \times 10^6$ cells/l); scenario III treating symptomatic patients (WHO stages III and IV); scenario IV treating patients with AIDS only; scenario V treating patients with CD4 cell count $< 200 \times 10^6$ cells/l only.

Results

As of December 2001, 292 patients had received HAART who met our inclusion criteria and constituted the treated arm of this study. The no-HAART group comprised 981 patients. Of these, seven patients did not have clinical or laboratory data and were, therefore, excluded from the analysis. Baseline demographic and clinical characteristics of the two groups are shown in Table 1. Age, gender, ethnicity and WHO stage did not differ in the two groups. More patients in the HAART group had a higher socioeconomic status. Prophylactic cotrimoxazole was used more frequently in the no-HAART group than in the HAART group. The HAART group had lower CD4 cell counts than did the no-HAART group. Mean

Table 1. Baseline demographic and clinical characteristics of the groups taking and not taking highly active antiretroviral therapy.

	HAART [No. (%)]	No HAART [No. (%)]	*P* value[a]
No. in group	292	974	
Age (years)			0.19
< 33)	145 (49.7)	441 (45.3)	
⩾ 33	147 (50.3)	533 (54.7)	
Gender			0.35
Male	159 (54.5)	502 (51.5)	
Female	132 (45.5)	472 (48.5)	
Population group			0.97
Black	170 (58.2)	573 (58.8)	
Mixed race	67 (23)	224 (23)	
White	55 (18.8)	177 (18.)	
Socioeconomic status			< 0.0001
High	149 (51)	346 (35.5)	
Low	143 (49)	628 (64.5)	
Year of initial care			< 0.0001**
1992–1996	0	588 (60.4)	
1997–2000	292 (100)	386 (39.6)	
Cotrimoxazole			< 0.0001
Yes	98 (33.6)	506 (52)	
No	194 (66.)	468 (48)	
WHO stage			0.09
I and II	157 (53.8)	541 (55.5)	
III	108 (37)	307 (31.5)	
IV	27 (9.2)	126 (13)	
CD4 cell count ($\times 10^6$ cells/l)			< 0.0001
< 200	102 (34.9)	447 (45.9)	
200–350	111 (38)	229 (23.5)	
> 350	79 (27.1)	298 (30.6)	

HAART, highly active antiretroviral therapy; WHO, World Health Organization.
*By chi-square test; **by Fisher exact test.

follow-up was longer in the HAART group (17.4 months) than in the no-HAART group (14.4 months) ($P = 0.0005$). The 415 baseline stage III diagnoses in this cohort consisted of 154 (37%) with pulmonary tuberculosis [median CD4 cell count 168×10^6 cells/l; interquartile range (IQR), 68–279], 206 (50%) with oral candidiasis or hairy leukoplakia (median CD4 cell count 166×10^6 cells/l; IQR, 61–276) and 55 (13%) with other stage III diagnoses (severe bacterial infections, weight loss > 10% of body weight, unexplained prolonged fever, unexplained chronic diarrhoea) (median CD4 cell count 201×10^6 cells/l; IQR 74–301).

In patients without prior AIDS, 10 new AIDS-defining illnesses occurred in the HAART group (2.8/100 PY) compared with 125 (12.9/100 PY) in the no-HAART group [unadjusted rate ratio (ARR), 0.22; 95% CI, 0.11–0.41; $P < 0.0001$]. Overall, AIDS-free survival proportion in the Kaplan–Meier analysis was significantly higher in the HAART compared with the no-HAART group ($P < 0.0001$), and remained significant when the analysis was stratified by baseline WHO stage, CD4 cell count or the different combinations of CD4 cell count and WHO stage (Fig. 1). After adjusting simultaneously for baseline differences in a Cox multivariate analysis, HAART conferred an independent protective benefit against risk of AIDS (ARR, 0.16; 95% CI, 0.08–0.31; $P < 0.0001$). Other variables associated with risk of AIDS were baseline WHO stage plus CD4 cell count (Table 2).

An analysis was carried out to calculate and compare the incidence of AIDS in the two groups, and to calculate the adjusted number of AIDS cases averted by HAART, stratifying patients by CD4 cell count, WHO stage, and the different combinations of WHO stages plus CD4 cell counts. Overall, adjusted number of AIDS cases averted was 10.8/100 PY (95% CI, 7.5–14.0), and this increased by advancing clinical stage of disease or immune suppression, with the largest number of adjusted cases averted in patients with WHO stage III (22.0/100 PY; 95% CI, 6.1–26.9) and CD4 cell count $< 200 \times 10^6$ cells/l (15.4/100 PY; 95% CI, 8.6–21.3) (Table 3).

A similar trend was observed when the analysis was carried out using death as an end-point. During the follow-up period, 15 deaths occurred in the HAART

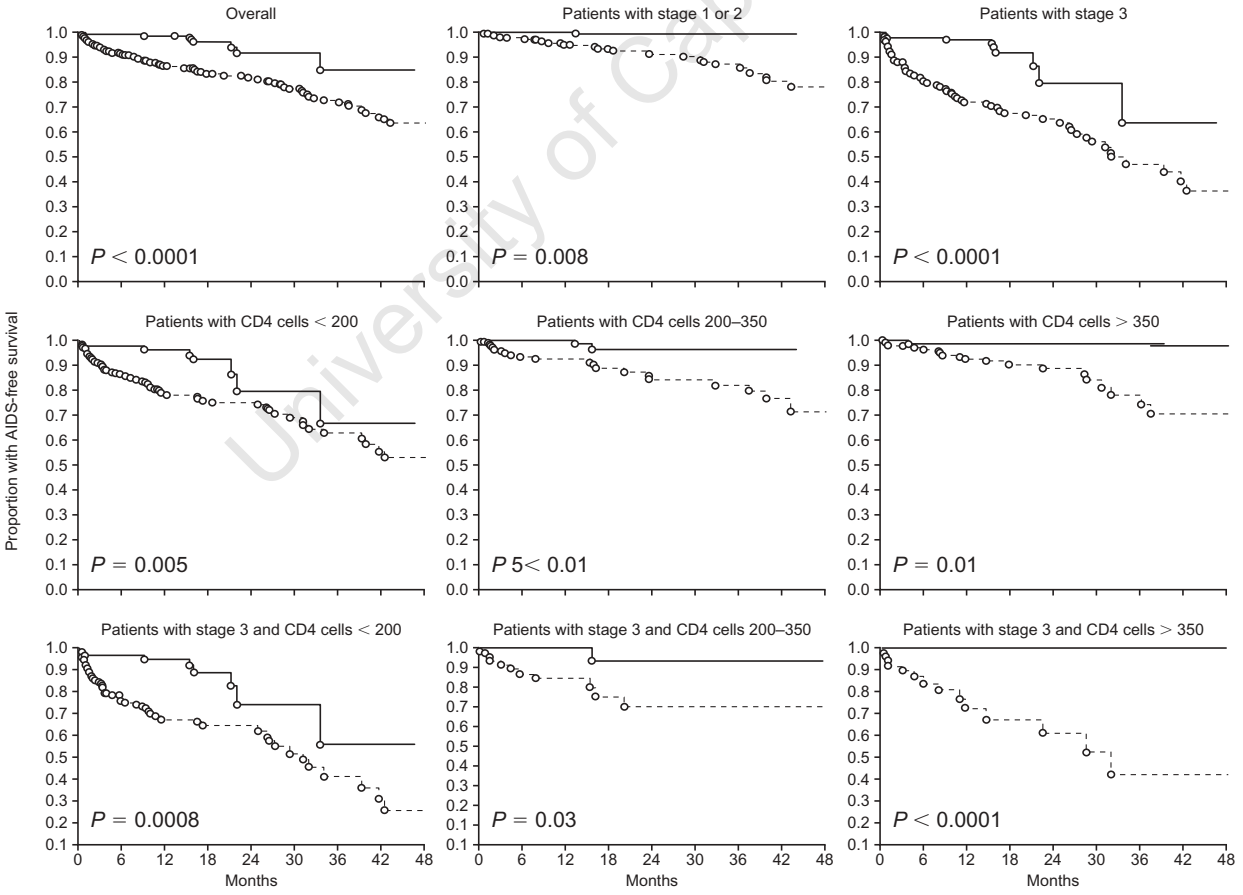

Fig. 1. Kaplan–Meier probabilities of AIDS-free survival for the group taking highly active antiretroviral therapy (solid line) and those not taking such therapy (dotted line). Stages are World Health Organization staging; CD4 cell counts given as $\times 10^6$ cells/l.

Table 2. Coxproportional hazards regression models for predictors of AIDS and death.

	Predictors of AIDS		Predictors of death	
	Univariate analysis RR (95% CI)	Multivariate analysis RR (95% CI)	Univariate analysis RR (95% CI)	Multivariate analysis RR (95% CI)
HAART				
Yes	0.23 (0.12–0.44)	0.16 (0.08–0.31)	0.14 (0.08–0.23)	0.10 (0.06–0.18)
No	1	1	1	1
Cotrimoxazole				
Yes	0.65 (0.47–0.97)	0.96 (0.66–1.39)	1.00 (0.80–1.25)	0.67 (0.53–0.85)
No	1	1	1	1
WHO stage				
I and II	0.22 (0.15–0.32)	0.23 (0.16–0.35)	0.14 (0.11–0.20)	0.15 (0.11–0.21)
III	1	1	0.45 (0.34–0.59)	0.48 (0.36–0.64)
IV	–	–	1	1
CD4 cell count ($\times 10^6$ cells/l)				
< 200	2.52 (1.61–3.95)	1.82 (1.13–2.91)	2.44 (1.80–3.30)	1.86 (1.36–2.55)
200–350	0.98 (0.57–1.71)	0.98 (0.56–1.72)	1.34 (0.94–1.91)	1.56 (1.09–2.26)
> 350	1	1	1	1
Initial year of care				
1992–1996	1.16 (0.81–1.65)	0.69 (0.47–1.01)	1.57 (1.24–1.99)	0.85 (0.66–1.09)
1997–2000	1	1	1	1
Age (years)				
< 33	0.88 (0.63–1.24)	0.96 (0.67–1.36)	0.78 (0.63–0.97)	0.84 (0.67–1.05)
$\geqslant 33$	1	1	1	1
Socioeconomic status				
High	1.02 (0.72–1.45)	1.17 (0.81–1.69)	1.09 (0.87–1.36)	0.95 (0.75–1.20)
Low	1	1	1	1

HAART, highly active antiretroviral therapy; WHO, World Health Organization.

group (3.6/100 PY) compared with 302 (26.4/100 PY) in the no-HAART group (ARR, 0.13; 95% CI, 0.08–0.23; $P < 0.0001$). Survival proportion was consistently significantly better in the HAART group than in the no-HAART group in the WHO stratified Kaplan–Meier analyses (Fig. 2). HAART was independently associated with significant reduction in death (ARR, 0.10; 95% CI, 0.06–0.18; $P < 0.0001$). Prophylactic cotrimoxazole, baseline CD4 cell count and WHO stage were also independently associated with risk of death (Table 2). The overall adjusted number of deaths averted was 23.8/100 PY (95% CI, 19.6–27.4) and, as in the AIDS analysis, also increased by advancing clinical stage of disease or immune suppression (Table 4).

In the analysis carried out to compare the relative impact of applying the WHO revised criteria and the other three scenarios defined above, the percentage of patients eligible for treatment from the total 974 no-HAART patients applying the revised WHO guidelines (56.7; 95% CI, 53.5–59.8) was significantly higher ($P < 0.05$) than that calculated for symptomatic WHO stage III or IV patients (44.5; 95% CI, 41.3–47.6), AIDS patients (12.9; 95% CI, 10.9–15.2) or patients with $< 200 \times 10^6$ cells/l CD4 cell counts (45.9; 95% CI, 42.7–49.1) (Table 5). However, the number of deaths averted per 100 PY using the WHO revised criteria (30.0/100 PY; 95% CI, 23.1–35.9) was not statistically different from that calculated applying other scenarios, except for patients with AIDS (cases averted 74.0/100 PY; 95% CI, 50.2–84.5). There was no significant advantage in terms of number of AIDS cases averted in treating symptomatic patients in comparison with treating patients meeting the revised WHO criteria (Table 3).

Discussion

This study is unique in that it compares the outcome of HIV disease in a group of patients receiving HAART with a comparison group without access to treatment, controlling for measurable confounding factors. Our findings address the identification of those at greatest need of immediate access to HAART in sub-Saharan Africa and are of practical relevance for the cost-effective use of scarce medical resources. HAART substantially reduced both progression to AIDS and deaths across all WHO stages plus CD4 cell counts. The magnitude of therapeutic benefit increased with advancing HIV disease progression. WHO clinical stage was a stronger predictor than CD4 cell count for both risk of AIDS and death.

Our comparative analysis of HAART initiation strategies demonstrated that a treatment threshold of clinical stage IV only would identify the lowest number (12.9%) of patients eligible for treatment but achieve the highest deaths/100 PY averted (74.0%). These patients would represent an appropriate target population for HAART where resources are severely con-

Table 3. AIDS incidence and cases averted.

	HAART				No HAART						
	No. patients	AIDS cases	PY	Incidence (per 100 PY)	No. patients	AIDS cases	PY	Incidence (per 100 PY)	Adjusted[a] rate ratio (95% CI)	*P* value	Adjusted[a] No. cases averted (95% CI)
Overall	264	10	357.5	2.8 (1.3–5.1)	848	125	967.4	12.9 (10.9–15.2)	0.16 (0.08–0.31)		10.8 (7.5–14.0)
WHO stage											
I and II	156	1	215.2	0.5 (0.01–2.6)	541	42	675.7	6.2 (4.5–8.3)	0.05 (0.01–0.41)	0.005	5.9 (2.7–8.2)
III	108	9	142.3	6.3 (2.9–11.7)	307	83	291.7	28.5 (23.3–34.0)	0.23 (0.11–0.49)	0.0002	22.0 (6.1–26.9)
CD4 cell count ($\times 10^6$ cells/l)											
< 200	81	8	115.2	6.9 (3.1–13.3)	361	76	395.8	19.2 (15.4–23.4)	0.20 (0.09–0.44)	< 0.0001	15.4 (8.6–21.3)
200–350	110	2	145.1	1.4 (0.2–4.9)	206	24	272.0	8.8 (5.7–12.8)	0.14 (0.03–0.72)	0.02	7.6 (1.6–12.4)
> 350	73	–	97.2	–	281	25	299.6	8.0 (5.5–12.1)	–		–
WHO stage plus CD4 cell count ($\times 10^6$ cells/l)											
Stage I and II plus CD4 cell < 200	24	–	33.5	–	186	21	235.7	8.9 (5.6–13.3)	–		–
Stage I and II plus CD4 cell 200–350	72	1	103.8	1.0 (0.02–5.2)	139	12	201.4	6.0 (3.1–10.2)	0.25 (0.02–0.48)	0.004	4.5 (1.6–10.00)
Stage I and II plus CD4 cell > 350	60	–	77.9	–	216	9	238.6	3.8 (1.7–7.0)	–		
Stage III plus CD4 cell < 200	57	8	81.7	9.8 (4.3–18.3)	175	55	160.1	34.4 (27.1–42.3)	0.31 (0.13–0.72)	0.006	23.7 (7.6–36.8)
Stage III plus CD4 cell 200–350	38	1	41.2	2.4 (0.1–12.9)	67	12	70.7	17.0 (9.1–27.7)	0.08 (0.01–0.83)	0.03	15.6 (1.6–27.4)
Stage III plus CD4 cell > 350	13	–	19.4	–	65	16	60.9	26.3 (15.8–39.1)	–		–

HAART, highly active antiretroviral therapy; WHO, World Health Organization; PY, person-years; CI, confidence interval.
[a]Adjusted for variables in Table 2.

strained. Targeting those with symptomatic HIV disease (WHO stages III and IV) would have resulted in a significantly lower number of individuals eligible for treatment (44.5%) than with implementation of the revised WHO criteria (56.7%) but would have resulted in comparable numbers of deaths/100 PY averted [40.8 (95% CI, 32.0–48.5) and 30.0 (95% CI, 23.1–35.9), respectively]. Implementation of therapy at a threshold of CD4 cell count $< 200 \times 10^6$ cells/l would also result in a significantly lower eligible proportion (45.9%) but would result in less deaths averted per 100 PY (29.6; 95% CI, 21.7–36.0) than treatment of symptomatic disease.

The decision of when to initiate HAART is multifaceted and depends on many factors, including available resources, the untreated prognosis, benefits of therapy, drug toxicity, and the need for long-term adherence. North American and European HIV treatment guidelines recommend initiating HAART for all patients with symptomatic disease, together with various CD4 cell count and viral load thresholds for asymptomatic individuals [15–17]. The WHO guidelines for scaling-up antiretroviral therapy in resource-poor settings included a CD4 cell count threshold of 200×10^6 cells/l for asymptomatic patients but excluded stage III disease with $> 200 \times 10^6$ cells/l, which was revised in 2003 to exclude stage III with a CD4 cell count $> 350 \times 10^6$ cells/l. In our cohort, 43% of stage III disease occurred at CD4 cell counts $> 200 \times 10^6$ cells/l. Individuals with stage III disease, regardless of their CD4 cell counts, had more AIDS events and deaths than those with stage I or II disease with a CD4 cell count of $< 200 \times 10^6$ cells/l. Oral candidiasis and oral hairy leukoplakia were the commonest stage III diagnoses (50%), with pulmonary tuberculosis (37%) and other stage III conditions (13%) occurring less frequently. CD4 cell counts did not differ significantly between these conditions, and comparable survival of patients with these conditions has been previously reported in our cohort [27]. Application of the initial WHO guidelines would have excluded 43% [20], and the revised WHO guidelines 21% [21], of stage III patients who would benefit from HAART. However, when CD4 cell count $< 200 \times 10^6$ cells/l is not used for access to HAART, and only symptomatic patients are treated, 41% of the patients with CD4 cell count $< 200 \times 10^6$ cells/l who may benefit from HAART would be excluded.

Several points should be considered in interpretation of our analysis. The observational design of our study is a limitation but, because of the undoubted benefits of HAART, a randomized placebo-controlled trial would not be a feasible alternative. In this none-randomized cohort study, there were some recognized but unavoidable confounders. The HAART group was largely self-selected and this may have resulted in a selection bias;

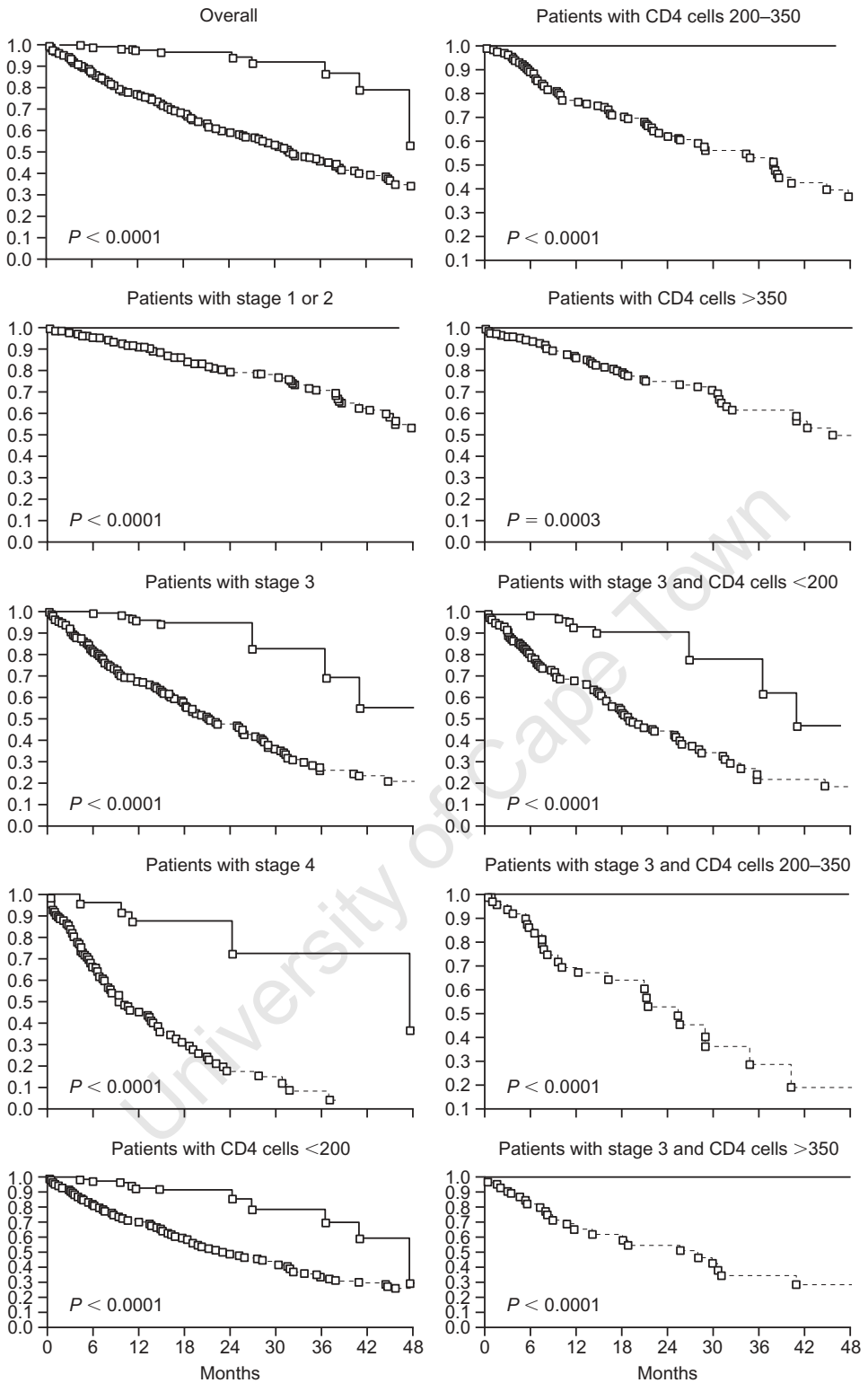

Fig. 2. Kaplan–Meier probabilities of survival of the group taking highly active antiretroviral therapy (solid line) and those not taking such therapy (dotted line). Stages are World Health Organization staging; CD4 cell counts given as $\times 10^6$ cells/l.

however, each group had a broad spectrum of immune suppression and clinical disease, which allowed for stratification of outcomes, and controlling for well-established prognostic indicators of disease progression. The median follow-up time was lower in those not receiving HAART, but this could not explain the higher event frequency noted in this group. The study population was attending a public sector health facility

Table 4. Death incidence and deaths averted.

	HAART				No-HAART						
	No. patients	Deaths	PY	Incidence (per 100 PY)	No. patients	Deaths	PY	Incidence (per 100 PY)	Adjusted[a] rate ratio (95% CI)	*P* value	Adjusted[a] No. cases averted (95%CI)
Overall	292	15	422.5	3.6 (1.9–5.8)	974	302	1,142.6	26.4 (23.9–29.1)	0.10 (0.06–0.18)	< 0.0001	23.8 (19.6–27.4)
WHO stage											
I and II	157	–	226.0	–	541	89	704.0	12.6 (10.3–15.3)	–		–
III	108	9	153.9	5.9 (2.7–10.8)	307	133	340.1	39.1 (33.9–44.5)	0.16 (0.08–0.34)	< 0.0001	32.8 (22.4–40.9)
IV	27	6	42.6	14.1 (5.3–27.9)	126	80	98.5	81.2 (71.7–88.0)	0.10 (0.04–0.30)	< 0.0001	74.0 (50.2–84.5)
CD4 cell count ($\times 10^6$ cells/l)											
< 200	102	14	156.9	8.9 (4.96–14.5)	447	177	502.7	35.2 (31.0–39.5)	0.16 (0.09–0.30)	< 0.0001	29.6 (21.7–36.0)
200–350	111	–	158.0	–	229	73	300.7	24.3 (19.5–29.5)	–		–
> 350	79	1	107.6	0.9 (0.02–5.1)	298	52	339.2	15.3 (11.7–19.6)	0.03 (0.004–0.26)	0.0012	14.8 (8.7–19.5)
WHO stage plus CD4 cell count ($\times 10^6$ cells/l)											
Stage I and II plus CD4 cell < 200	24	–	34.6	–	186	38	247.1	15.4 (11.1–20.5)	–		–
Stage I and II plus CD4 cell 200–350	72	–	110.5	–	139	32	209.3	15.3 (10.7–20.9)	–		–
Stage I and II plus CD4 cell > 350	61	–	80.9	–	216	19	247.6	7.7 (4.7–11.7)	–		–
Stage III plus CD4 cell < 200	57	8	87.4	9.2 (4.1–17.3)	175	83	190.9	43.5 (36.3–50.8)	0.24 (0.11–0.56)		33.1 (16.0–45.2)
Stage III plus CD4 cell 200–350	38	–	46.2	–	67	26	74.3	35.0 (24.4–47.1)	–		–
Stage III plus CD4 cell > 350	13	1	20.3	4.9 (0.1–24.9)	65	24	74.9	32.0 (21.7–43.8)	0.15 (0.01–0.61)	0.012	27.2 (8.5–43.4)
Stage IV plus CD4 cell < 200	21	6	34.8	17.2 (6.6–33.7)	86	56	64.7	86.6 (75.3–93.5)	0.11 (0.04–0.34)	< 0.0001	77.0 (49.7–89.8)
Stage IV plus CD4 cell 200–350	1	–	1.3	–	23	15	17.2	87.2 (63.6–98.5)	–		–
Stage IV plus CD4 cell > 350	5	–	6.5	–	17	9	16.6	54.2 (27.8–77.0)	–		–

HAART, highly active antiretroviral therapy; WHO, World Health Organization; PY, person-years; CI, confidence interval.
[a]Adjusted for variables in Table 2.

Table 5. Comparison of the projected number of patients qualifying for highly active antiretroviral therapy (HAART) and the deaths averted by the application of five different HAART initiation scenarios to the 974 patients in the no-HAART group.

	HAART initiation criteria[a]				
Variable	Scenario I	Scenario II	Scenario III	Scenario IV	Scenario V
No of patients eligible for treatment	552	487	433	126	447
Percentage of patients eligible for treatment	56.7 (53.5–59.8)	50 (46.8–53.2)*	44.5 (41.3–47.6)*	12.9 (10.9–15.2)*	45.9 (42.7–49.1)*
Death rate without HAART	36.6 (33.0–40.3)	37.5 (33.3–41.7)	48.6 (43.8–53.3)	81.2 (71.7–88.0)	35.2 (31.0–39.5)
Death rate with HAART	6.5 (3.7–10.5)	8.3 (4.7–13.8)	7.6 (4.3–12.3)	14.1 (5.3–27.9)	8.9 (4.96–14.5)
Relative risk of death	0.18 (0.11–0.30)	0.23 (0.13–0.39)	0.16 (0.09–0.27)	0.10 (0.04–0.30)	0.16 (0.09–0.30)
Deaths averted per 100 PY HAART	30.0 (23.1–35.9)	28.9 (20.3–36.3)	40.8 (32.0–48.5)	74.0 (50.2–84.5)*	29.6 (21.7–36.0)

[a]Scenario I revised WHO treatment guidelines; scenario II WHO previous treatment guidelines; scenario III treating symptomatic patients (WHO Stage III and 4); scenario IV treating patients with AIDS only; scenario V treating patients with $< 200 \times 10^6$ cells/l only. Death rates for each scenario were calculated from Table 4.
*Values significantly differ from scenario I ($P < 0.05$).

and may not be representative of the total South African HIV-infected population; however, it would resemble those who are most likely to access HAART in a national treatment programme. We did not adjust our results for viral load, as it was not available for the no-HAART group. However, while baseline CD4 cell count and clinical stage were predictive of on-HAART progression in a large multicentre analysis, only viral loads $> 10^5$ copies/ml were associated with increased progression rate [8]. Survival of our untreated cohort was not dissimilar from that of the US Multi-Center AIDS Cohort Study [36], and better than elsewhere in Africa [22,23,37,38], probably because of the availability of rifampicin-based tuberculosis therapy, cotrimoxazole prophylaxis and treatment of common opportunistic infections. Death rates in our on-HAART group were comparable to those reported by Egger *et al.* [8]; however, cause-specific mortality was not available, and, therefore, it was not possible to ascertain whether all deaths reported in both cohorts studied were HIV related. The clinical benefits of HAART in this indigent African population appear to be of similar magnitude to those in developed world settings. Low socioeconomic status is not a predictor of poor adherence to HAART [39], and indigent African patients have been shown to be able to adhere to therapy and achieve high rates of viral suppression [39–49]. The low number of individuals accessing HAART in resource-poor settings is a consequence of economic factors, particularly the prohibitive cost of drugs, rather than any medical rationale.

We have quantified the impact of HAART initiated at various thresholds and shown a graded response: greatest in those with AIDS and lowest in asymptomatic disease with preserved CD4 cell counts. The combination of the number of AIDS and deaths averted by varying strategies and the proportion of individuals eligible for treatment at different initiation thresholds are useful data for HAART programme planning and cost-effectiveness modelling. Where medical resources are limited, it is appropriate to treat first those who would benefit most from HAART. The superiority of WHO clinical stage over CD4 cell count for identifying those who will benefit most from HAART allows therapy to be targeted to symptomatic individuals who are already likely to be seeking care within the health system. Where resources are less constrained, CD4 cell counts can be utilized to identify asymptomatic patients at high risk of progression to AIDS and death, who may also benefit from HAART. Implementation of the 2003 revised WHO guidelines in sub-Saharan Africa could result in a significantly larger number of eligible individuals but with lower impact on mortality than an alternative strategy based on clinical parameters.

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Rapid scale-up of a community-based HIV treatment service

Programme performance over 3 consecutive years in Guguletu, South Africa

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Background. Despite rapid expansion of antiretroviral therapy (ART) in sub-Saharan Africa there are few longitudinal data describing programme performance during rapid scale-up.

Methods. We compared mortality, viral suppression and programme retention in 3 consecutive years of a public sector community-based ART clinic in a South African township. Data were collected prospectively from establishment of services in October 2002 to the censoring date in September 2005. Viral load and CD4 counts were monitored at 4-monthly intervals. Community-based counsellors provided adherence and programme support.

Results. During the study period 1 139 ART-naïve patients received ART (161, 280 and 698 in the 1st, 2nd and 3rd years respectively). The median CD4 cell counts were 84 cells/µl (interquartile range (IQR) 42 - 139), 89 cells/µl (IQR 490 - 149), and 110 cells/µl (IQR 55 - 172), and the proportions of patients with World Health Organization (WHO) clinical stages 3 and 4 were 90%, 79% and 76% in each sequential year respectively. The number of counsellors increased from 6 to 28 and the median number of clients allocated to each counsellor increased from 13 to 33. The overall loss to follow-up was 2.9%. At the date of censoring, the Kaplan-Meier estimates of the proportion of patients still on the programme were 82%, 86% and 91%, and the proportion who were virally suppressed (< 400 copies/ml) were 100%, 92% and 98% for the 2002, 2003 and 2004 cohorts respectively.

Conclusions. While further operational research is required into optimal models of care in different populations across sub-Saharan Africa, these results demonstrate that a single community-based public sector ART clinic can extend care to over 1 000 patients in an urban setting without compromising programme performance.

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In 2003 the World Health Organization (WHO) and the United Nations AIDS Organization (UNAIDS) launched a strategy to extend antiretroviral treatment (ART) to 3 million people living with HIV/AIDS in low- and middle-income countries by the end of 2005.[1] As a result of this strategy there are now over 500 000 people on ART in sub-Saharan Africa, where the burden of disease is greatest. This reflects a 3-fold increase in the number of people receiving treatment in the region during the 12 months up to June 2005.[2] With this dramatic expansion in services, concerns have been raised that expanding access to ART in resource-poor settings will lead to 'antiretroviral anarchy' characterised by poor adherence to therapy, widespread viral resistance to medications and ultimately poor clinical outcomes.[3]

To date, a number of programmes have demonstrated the feasibility of providing ART services on a relatively small scale,[4-10] and have shown that in these instances clinical and virological outcomes in these programmes parallel outcomes in Europe and North America.[11,12] A recent article by Severe *et al*.[13] makes an important contribution to the published literature on the scale-up of access to ART in resource-limited settings. The results of the first 1 000 patients treated in Port-au-Prince, Haiti, with limited infrastructure and staff are remarkable compared with those from First-World settings. After 1 year of therapy, 87% of adults and 98% of children were still alive. An editorial in the same journal[14] called for accelerated enrolment in both urban and rural settings. But despite the rapid expansion of ART services in sub-Saharan Africa, there are few documented experiences of how the expansion of ART services over time may affect their quality. As ART programmes increase the numbers of patients served, there are parallel increases in the burden on staff, affecting each category of health care provider. The increased burden on health providers associated with scale-up may result in reduced time, on average, spent in the care of each patient in

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the service. Similar phenomena, with increases in patient load associated with reductions in programmatic success, have been documented in a number of other primary care services, including the diagnosis and management of tuberculosis and sexually transmitted infections.[15-17] In this light it is possible that the good programmatic outcomes – including high rates of virological suppression and retention in services along with low mortality – that have been achieved by small, focused ART services, may be difficult to maintain as programmes grow in size.

The question of how programmatic outcomes in antiretroviral services are influenced by rapid increases in patient load has significant implications for the scale-up of HIV care and treatment services in sub-Saharan Africa. While there is a wide range of different approaches to delivering ART services, there are few insights into the service delivery models that can best maintain optimal patient care and programmatic outcomes. For example, if programmes with large patient loads have difficulty maintaining the high levels of viral suppression and patient retention and low levels of mortality that can be achieved with smaller services, then multiple small-scale services may be preferable to fewer, larger services for delivering ART to a specific population. Insights into these questions of programme expansion and optimal patient load are urgently required to inform health systems and policies for ART scale-up in resource-limited settings.

In this light, we reviewed changing patterns in virological outcomes, patient survival and programme retention among patients attending a public sector community-based ART programme in a South African township. The programme was established in September 2002 and rapidly expanded its services, enrolling 1 139 patients by October 2005.

Methods

Setting

Guguletu Clinic is situated in the urban Nyanga district of Cape Town, which has an estimated population of 350 000 and is served by 10 primary care HIV clinics[18] that comprised the patient referral base. The district is socially deprived with an estimated 57% unemployment rate and with 81% of households living in informal dwellings,[19] a tuberculosis notification rate of 1 026/100 000,[20] and an antenatal HIV-1 seroprevalence rate of 28%, which is among the highest in the province.[21] The *Usapho Lwethu* (Our Family) ART programme is a dedicated ART clinic based at the Guguletu Community Health Centre, Cape Town.[22] A pilot programme was started in 2002 with funding support from an international donor, and the expansion of services in subsequent years has been supported by a grant from the Global Fund to Fight AIDS, Tuberculosis and Malaria.

ART programme

Enrolment into the programme follows the National Ministry of Health ART guidelines,[23] which are based on the 2002 WHO recommendations for scaling up ART in resource-poor settings.[24] The medical criteria for adult ART eligibility include those with a prior AIDS diagnosis or a blood CD4 cell count < 200 cells/µl, and for children, paediatric stage III disease or a CD4 cell percentage < 20%.

Following referral from a primary care clinic, the standard schedule of visits was as follows: screening visit (week 0), screening blood tests (week 2), treatment initiation (week 4) and treatment follow-up (weeks 8, 12, and 20, and 16-weekly thereafter). At the screening visit, a treatment-readiness evaluation was completed and a 4-week supply of co-trimoxazole was dispensed to patients with less than 200 CD4 cells/µl, with pill counts at 14 and 28 days to assess adherence. Dapsone was used as an alternative for those with co-trimoxazole intolerance. Patients were assessed by a doctor for symptomatic HIV-associated disease. First-line ART comprised stavudine, lamivudine plus a non-nucleoside reverse transcriptase inhibitor (efavirenz or nevirapine). The second-line regimen comprised lopinavir/ritonavir, zidovudine and didanosine. A secure supply of medication was maintained by the local health authority throughout the study period and all treatment was supplied free of charge. In addition to the scheduled clinic appointments, patients had open access to the clinic for medical problems.

Therapeutic counsellors

At the screening visit patients were allocated to a community-based 'therapeutic counsellor' living in the Guguletu area. These community-based counsellors, the majority of whom are living with HIV/AIDS, provide ongoing counselling support, addressing psychosocial issues and reinforcing the need for high levels of treatment adherence. Counsellors were responsible for treatment-readiness group information sessions carried out twice weekly in a local community hall, clinic-based adherence reinforcement sessions and home visiting.

Laboratory monitoring

Plasma HIV load and blood CD4 cell count were performed at week 2 and 4-monthly after commencing ART. Toxicity monitoring comprised a full blood count and liver function tests performed at week 2, and subsequently at 4-monthly intervals. All laboratory tests were performed on site by a single laboratory technician in a self-contained laboratory located in a modified shipping container. Plasma HIV-1 load was measured using the Versant HIV-1 RNA 3.0 assay performed on a 340 bDNA analyser (Bayer Diagnostics, Tarrytown, NY, USA) with a lower limit of detection of 50 RNA copies/ml. Blood CD4 cell counts were measured using

flow cytometry (Becton Dickenson FACSCount, New Jersey, USA). Internal quality assurance (QA) and interlab QA were performed for the viral load and CD4 measurements and full blood counts and CD4 measurements were subject to additional external QA.

Data sources

Structured clinical records were maintained on all patients screened on entry to the ART programme and this information together with laboratory results was regularly transferred to an electronic database. The Research Ethics Committee of the University of Cape Town approved the use of patient information from this service for programme evaluation, and as part of this, all enrolled patients gave written informed consent for anonymous clinical data to be recorded and analysed.

Statistical analysis

Data were analysed using Stata version 9.0 (College Station, Texas, USA). Three annual cohorts were defined as patients initiating ART during the 12 months from 1 September 2002 (the 2002/2003 cohort), 2003 (the 2003/2004 cohort) and 2004 (the 2004/2005 cohort). The demographic profile and baseline immunological and virological characteristics of the cohorts were compared using Wilcoxon's rank-sum and chi-square tests for medians and proportions, respectively; trends across annual cohorts were assessed using Cuzick's non-parametric trend test for medians and Cochrane-Armitage tests for proportions. The retention of patients in the programme was compared across annual cohorts using Kaplan-Meier analyses with log-rank tests. All statistical tests are 2-sided at $\alpha = 0.05$.

Results

Programme entry

During the first 3 years of the service, 1 510 patients were referred to the programme for ART, of whom 1 139 were commenced on treatment. Of the 371 not starting ART, 155 (42%) did not meet entry qualifications or received therapy elsewhere, 146 (39%) were awaiting treatment initiation at the censoring date and 70 (19%) died before starting ART. The pre-ART death rate was 34.6/100 patient-years (confidence interval (CI): 27 - 43) and did not vary significantly between the annual cohorts. Recruitment increased steadily during the study period (October 2002 - September 2005), with greater numbers of children and pregnant women entering the programme in the second and third years (Table I). There was a significant trend for individuals recruited into the programme to have less advanced HIV in the second and third years as demonstrated by higher median CD4 count, lower $\log_{10}$ mean viral load and a lower proportion with an AIDS diagnosis (Table I). The proportion of pregnant women entering the programme who had AIDS was low (5%) compared with non-pregnant adults (30%). The CD4 count of children at programme entry (median 540 cells/µl, interquartile range (IQR) 244 - 906) was significantly higher than that of adults (101 cells/µl, IQR 244 - 906). As further treatment sites were initiated elsewhere in South Africa, an increasing number of patients were transferred in and out of the programme on ART.

Staffing and physical infrastructure

The programme commenced with a staff of 1 doctor, 1 nurse and 8 counsellors. At the end of the study period staffing

Table I. Baseline characteristics of 1 139 patients starting ART between October 2002 and September 2005

	2002/3	2003/4	2004/5	*p*-value
Number starting ART	161	280	698	
Median age (yrs)	33	32	32	0.19
Age range (yrs)	7 - 58	1 - 54	1 - 64	
Female (*N* (%))	114 (74.5)	193 (75.7)	479 (65.4)	0.002
Pregnant at entry (*N*)	1	39	80	0.06
Children < 14 years (*N*)	1	3	69	< 0.001
Median CD4 (cells/µl (IQR))	84 (42 - 139)	89 (49 - 149)	110 (55 - 172)	< 0.001
Median $\log_{10}$ viral c/ml (IQR)	4.98 (4.58 - 5.33)	4.87 (4.50 - 5.23)	4.72 (4.25 - 5.14)	< 0.001
WHO stage 4 (*N* (%))	68 (44.4)	64 (25.1)	157 (21.5)	< 0.001
WHO stage 3 (*N* (%))	69 (45.1)	140 (54.9)	397 (54.2)	
WHO stages 1 and 2 (*N* (%))	16 (10.5)	51 (20.0)	178 (24.3)	
Transfers in/out (*N*)	0/1	0/39	32/80	
Died on treatment (*N*)	21	22	35	
Lost to follow-up (*N*)	8	17	8	

IQR = interquartile range.

had increased to 4 doctors, 3 nurses, 1 pharmacist and 28 counsellors (Fig. 1). There was a high turnover of the professional staff employed by the local provincial authority, with frequent use of temporary staff to provide services. In contrast, there was very low turnover of counsellors, who constitute approximately 80% of all staff employed by the programme. The number of clients allocated to each counsellor increased over time, with a median of 13, 19, and 33 patients per counsellor during 2002/3, 2003/4 and 2004/5, respectively. In terms of infrastructure, the ART service started in a single clinic room within the local health facility. Increasing patient numbers necessitated a move to an on-site prefabricated cabin in 2004 and to a self-contained building within the clinic grounds in 2005.

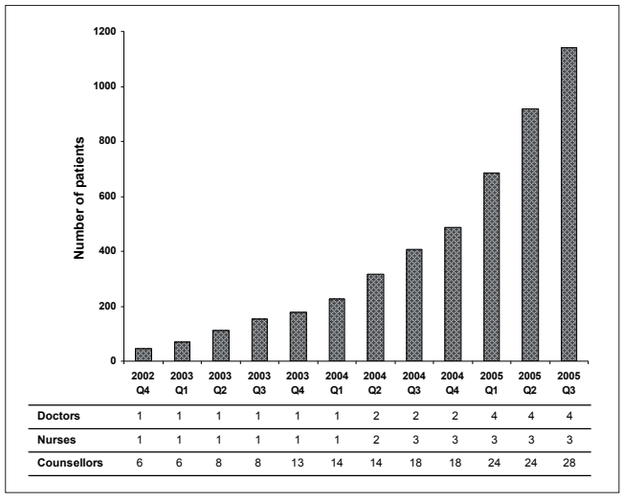
Programme retention

One hundred and one patients were lost to the programme, and 78 patients (7%) died after starting ART, with 63% of deaths occurring in the first 90 days of ART.

Thirty-three patients (3%) were lost to follow-up during the study period. The Kaplan-Meier estimate of proportion lost to programme at 1 year did not differ significantly between the 3 annual cohorts (Fig. 2).

Virological outcomes

The median proportions of viral loads < 400 copies/ml and < 50 copies/ml at all monitoring time points were 95% and 82%. At the censoring date, the 2002/3, 2003/4 and 2004/5 cohorts had 100%, 92% and 98% of viral loads < 400 copies/ml and 88%, 67% and 85% < 50 copies/ml respectively (Fig. 3, A and B). During the study period 21 patients had 2 consecutive viral loads > 1 000 copies/ml (6, 11 and 5 in each sequential annual period) and 17 patients were changed from first- to second-line regimen (6, 8 and 3 in each of the sequential annual periods).



	2002 Q4	2003 Q1	2003 Q2	2003 Q3	2003 Q4	2004 Q1	2004 Q2	2004 Q3	2004 Q4	2005 Q1	2005 Q2	2005 Q3
Doctors	1	1	1	1	1	1	2	2	2	4	4	4
Nurses	1	1	1	1	1	1	2	3	3	3	3	3
Counsellors	6	6	8	8	13	14	14	18	18	24	24	28

Fig. 1. The cumulative number of patients recruited onto the antiretroviral programme together with number of doctors, nurses and counsellors in each quarter-year period between October 2002 and September 2005.

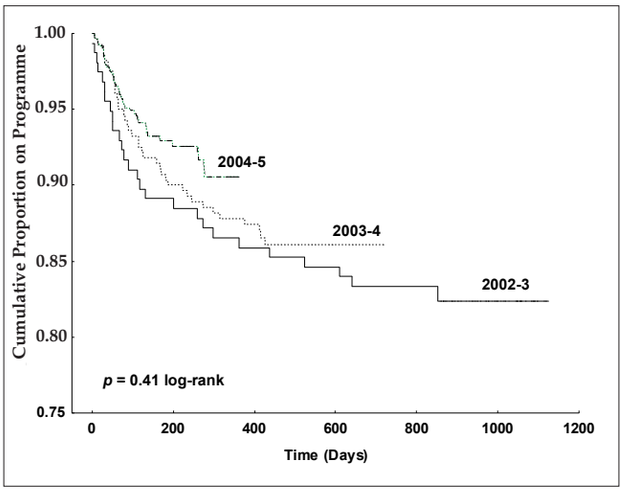


Fig. 2. The cumulative proportion of individuals remaining on ART for each of the 3 annual cohorts (Kaplan-Meier analysis). The survival proportions of the cohorts do not differ significantly (log-rank test).

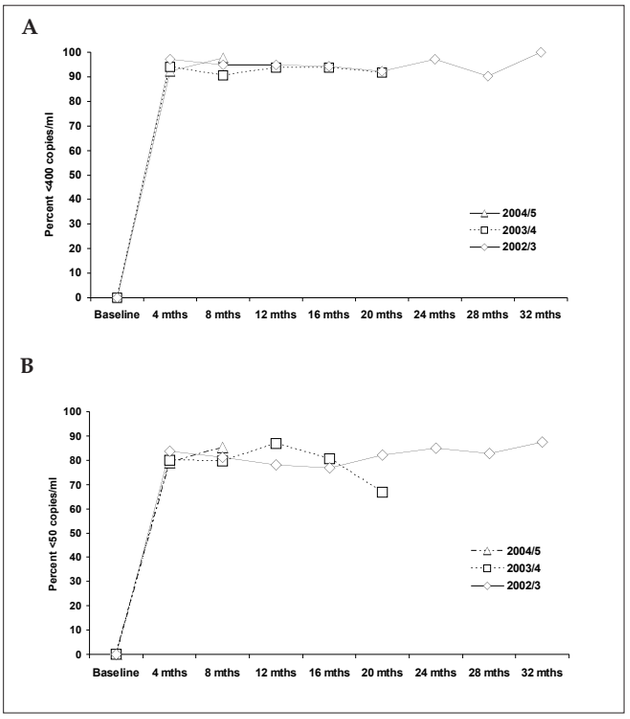


Fig. 3. A: The proportion of individuals in each annual cohort with < 400 HIV copies per ml of plasma at each 4-month monitoring time point. B: The proportion of individuals in each annual cohort with < 50 HIV copies per ml of plasma at each 4-month monitoring time point.

Discussion

This is one of longest-running public-sector community-based ART programmes in sub-Saharan Africa, allowing a unique assessment of trends during a period of increasing recruitment

to more than 1 000 patients at a single clinic. On-treatment virological suppression rates were excellent, were sustained over the 3-year period and compare very favourably with other published treatment programme outcomes in Africa,[4-10] Europe and North America.[25] These results show that key programmatic outcomes can be maintained during a period of rapid expansion of services.

While the experience of this programme in rapidly expanding services is encouraging, these results should be interpreted with the following caveats. Recruitment to the programme was restricted to individuals living within a demarcated urban catchment area. While this geographical proximity may be similar to that of other peri-urban townships where large populations access health care, this model may not be generalisable to rural and other settings where populations are more dispersed. Furthermore the median pre-ART period of follow-up was similar for each of the yearly cohorts and therefore pre-ART mortality rates could be compared. However, the median on-ART follow-up varied and was shorter for the later cohorts. Therefore, while the short-term results were comparable, the long-term results seen in the 2003/4 cohort may not necessarily be achieved by those accessing therapy later.

The low proportion of males together with the advanced clinical stage of non-pregnant adults accessing this programme is of concern, as this may be an indication that ART coverage is still inadequate at a population level. Patients generally accessed the programme when their disease was advanced, which is reflected by high mortality in the pre-ART and early treatment phase. The high mortality and morbidity of those who present with very advanced illness disproportionately utilises medical resources around the time of ART initiation.[26] Clinic recruitment could be increased if the programme were accessed earlier. There was a trend towards an increase in median CD4 cell count over the study periods, which was more marked in the third year. It would be encouraging if earlier presentation represented a decrease in the backlog of advanced HIV infection in the population; however the increase in median CD4 cell count was not paralleled by a decrease in pre-ART mortality, which remained unacceptably high. The higher median CD4 cell count was largely explained by an increase in non-traditional recruitment of pregnant women and children. Pregnant women were referred after accessing voluntary counselling together with CD4 cell count testing at the local maternal-obstetric service and had markedly less symptomatic HIV disease. The increasing number of children reflected an increase in collaboration with paediatric HIV services resulting in increased referrals from hospital-based specialist units to community care, and children had significantly higher baseline CD4 cell counts than adults.

To date, physical infrastructure has not been identified as a key barrier to implementation of ART access in the context of small-scale services.[27] However our experience has been that physical space is a critical constraint in the expansion of this programme, as the clinic had to be relocated twice in 3 years, including the development of an off-site location for pre-treatment counselling. In addition, the positioning of a dedicated laboratory in a shipping container on site enabled an efficient monitoring service, avoiding the need for sample courier services and expediting access of results to medical staff. The on-site laboratory was economically viable when monitoring > 1 000 patients on ART (data not shown) and could be used for similar large single clinics or geographically clustered clinics in both urban and rural settings serving 1 000 - 10 000 patients. An increasing number of patients were transferred from and to other treatment centres during year 3. As ART programmes mature there will be an increasing need for tracking systems, which can enable efficient transfer of patients between treatment centres.

Although programme acceptability was not specifically measured as part of routine clinical services, the low rate of loss to follow-up observed may reflect levels of client satisfaction. Shortage of human resources, in particular nurses, has been identified as a major operational constraint to treatment access.[27] The specific model of care reported in this study was a dedicated ART community clinic, relying on a high ratio of lay counsellors to clients. The community-based counsellors supplied continuity and served as the major conduit for ongoing interaction between the programme and clients. The allocation of a personal 'therapeutic counsellor' to each client on entry into the programme facilitated the high levels of treatment adherence (contributing to virological suppression rates) and retention in the programme. In contrast to the health professionals, they are readily accessible and their utilisation results in employment opportunities for individuals living with HIV/AIDS in a community with high unemployment.

In summary, this analysis of a community-based, public-sector ART service demonstrates that a single urban public clinic can supply and monitor ART to more than 1 000 patients, without compromising programme performance during rapid scale-up. This finding bodes well for the expansion of ART services in resource-limited settings

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Performance of a trained traditional bonesetter in primary fracture care

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Background. In developing nations traditional bonesetters (TBSs) play a significant role in primary fracture care. However, despite high patronage the TBS remains an untrained quack whose practice is often associated with high morbidity. This study evaluated the performance of a trained TBS in primary fracture care.

Methods. Between 2002 and 2004 a prospective study was undertaken comparing the performance of a trained TBS with that of an untrained TBS at two separate locations. The two centres selected were both popular in traditional bone setting. A 1-day instructional course was given to the TBS at Afuje study centre, while the TBS at Ogua control centre received no instruction. The outcome of treatment of tibial shaft fractures at the two centres was evaluated and compared to assess the success of the course.

Results. There was a considerable decrease in the rate of gangrenous limbs, infection, non-union and malunion at the trained TBS centre compared with the untrained TBS centre (2.5% v. 10%, 5% v. 12.5%, 7.5% v. 15%, and 20.0% v. 30%, respectively). The observed difference between the trained and untrained TBSs was statistically significant ($p < 0.05$).

Conclusion. It appears that training TBSs can reduce morbidity rates following TBS treatment.

S Afr Med J 2006; **96:** 320-322.

In most developing nations traditional bonesetters (TBSs) still play a significant role in primary fracture care.[1-12] They are highly patronised by local people. Over 70.0% of the rural population relies on the TBS for fracture treatment. While many fractures do heal properly with traditional treatment, bonesetters often do not appreciate the dangers of the complications arising from their practices.[2-10]

It is generally believed that TBSs are not trainable, but I have observed that some are willing to learn new methods of treatment to improve their services.[1] At least they could be trained as plaster technicians and be incorporated into primary health care services. I therefore conducted a 2-year prospective study to evaluate the performance of a trained TBS in primary fracture care in a developing country.

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Methods

In the period between January 2002 and December 2004 I evaluated the performance of a trained TBS at Afuje TBS centre, a 75-bed facility for the traditional treatment of fractures.

The centre is located in the rural area of Owan East Local Government Area (LGA), Edo North Senatorial district of Nigeria, where most people are subsistence farmers. The TBS centre is situated in a remote area, where there is no orthodox orthopaedic practice. The chief TBS has three sons who help him to set bones. Two of the sons are university undergraduates. The centre enjoys the services of a trained nurse and a general practitioner, and uses an X-ray facility situated about 5 km away for diagnosis and follow-up. The general practitioner, who is in private practice, often co-operates with the TBS and does osteoclasis and open reduction without fixation for cases of malunion and delayed union as requested by the TBS. After the skin wounds have healed, the patients are sent back to the TBS centre for subsequent treatment of the fracture. Most cases of fresh fractures in the non-specialist private clinics are directed to the TBS centres for treatment. Fracture victims find the TBS method attractive because it obviates the use of implants, which most of the local people dislike. The nurse at the TBS centre visits the centre often to sell analgesics, antibiotics and haematinics. She is occasionally requested to immunise patients against tetanus. The Afuje TBS centre, a well-organised 'tradorthopaedic' clinic, was used as the study centre (A).

Another popular TBS centre in a similar rural setting at Ogua in Esan West LGA was used as the control for the study (centre B). The two selected centres used similar conservative methods of treating fractures and were both reputable traditional bone setting practitioners with good patronage within and outside their LGAs. The co-operation of the TBSs at the centres was won with the assistance of my photographer, who at various times had personally had fractures treated at these centres.

I was able to give a 1-day instructional course to the TBS at the Afuje study centre (A) on safe conservative treatment of fractures with regard to diagnosis, patient selection, basic principles of fracture treatment, prevention of complications including transmission of HIV infection, referral services and outcome of treatment. Centre B received no instruction.

A 2-year prospective study of the outcome of fracture treatment at the two centres was conducted soon after the course between 2002 and 2004 in order to assess the success of the course. The outcome of the treatment of tibial shaft fractures at the centres was assessed. The chi-square method was used to test for significance of the outcome of the study.

Results

Between January 2002 and December 2004 I studied the outcome of 40 tibial shaft fractures treated in centre A. There were 40 similar tibial shaft fractures in centre B. In both centres the male-to-female ratio was 3:1; the ages of the patients ranged from 20 years to 65 years with a median age of 30 years, and the distribution of the pattern of fractures between the groups was fairly similar.

The outcome of the treatment of tibial shaft fractures is shown in Table I.

Table I. Outcome of the treatment of tibial shaft fractures at Afuje (A) and Ogua (B) TBS centres

Outcome	No. (%) Centre A	Centre B
Acceptable union	19 (47.5)	5 (12.5)
Malunion	8 (20.0)	12 (30.0)
Non-union	3 (7.5)	6 (15.0)
Delayed union	7 (17.5)	8 (20.0)
Post-traumatic osteomyelitis	2 (5.0)	5 (12.5)
Limb gangrene	1 (2.5)	4 (10.0)
Total	40 (100)	40 (100)

There was no death in either of the centres. The median duration of stay in centre A was 4 months (range 2 - 12 months) for patients with tibial shaft fractures. In centre B the median duration of stay was 8 months (range 2 - 18 months). The rate of voluntary discharge was 25% (10 patients) from centre A and 50% (20 patients) from centre B. Voluntary discharge was usually due to dissatisfaction with the outcome of treatment, such as non-union, delayed union, infection or gangrene. In both centres the TBS no longer used the same blade for more than one patient for scarification. Each patient bought his or her own personal blade for this purpose.

Most of the patients studied had received their initial treatment in an orthodox hospital before discharging themselves and seeking treatment from the TBS. In most cases this decision was due to pressure from senior members of the victim's family.

Discussion

The instruction received by the TBS on safe application of a splint in tibial shaft fracture appeared to result in a marked decrease in iatrogenic limb gangrene in centre A, where there was 1 case of extremity gangrene compared with 4 cases in centre B (control). TBSs often use splits made from split bamboo or strips of wood tightly bound around the limb,[1] which may not be removed when pain increases after immobilisation. A compartment syndrome with its permanent sequelae and gangrene may result. For gangrene, amputation is the only possible treatment. Death may result from complications such as tetanus and septicaemia.[6]

These problems are due to a lack of awareness of the dangers of tight splints among TBSs. The TBS has traditionally been considered secretive, and it was difficult to teach them how

complications could be prevented.[3,6] However, with the co-operation of the modern TBS it was possible to arrange 1-day instructional courses on the safe fracture treatment. This has resulted in a considerable decrease in the rate of gangrenous limbs, infection, non-union and malunion. There has been a significant increase in the rate of acceptable union among patients treated by the trained TBS compared with the untrained TBS ($p < 0.05$).

The morbidity associated with fracture treatment in an untrained TBS centre is high, and the median period of 8 months' stay in such centres is distressing in terms of man-hours and productivity lost. Training reduced the median period of stay to 4 months for tibial shaft fractures in study centre A.

If voluntary discharge from an untrained TBS centre implies a dismal outcome of treatment, the rate of treatment failure at such centres is very high at 50%. This suggests that people's confidence in the TBS is misplaced. Training reduced the rate of voluntary discharge by half. Voluntary discharge of fracture victims in a developing nation from one treatment facility to another is a complex phenomenon. Voluntary discharge from an orthodox hospital to a TBS has, for example, been blamed on an external locus of control in decision-making and not failure of the orthodox hospitals.[11] The high rate of return to orthodox care suggests that people's confidence in the TBS is highly misplaced and their internal locus of control in decision-making is reawakened by TBS failure.[5,11,12] Over 80% of the patients who had been treated by an untrained TBS had complications.[2-15] This is not surprising as their treatment consists of the application of herbs and wrapping of the fractured limbs in short traditional splints and cloth[14] after inadequate reduction and stabilisation, and rehabilitation.

Conclusions

It was been established that the modern TBS is willing to co-operate with the orthodox orthopaedic practitioner,[1,16] and the results of this study showed that training improves performance of the TBS. I recommend that the TBS should be trained as a traditional orthopaedic attendant (TOA) for effective primary fracture care in developing countries.

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Cost of a dedicated ART clinic

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Background. The provision of antiretroviral therapy (ART) is being rolled out across South Africa. Little evidence exists on the cost of running clinics for ART provision.

Objectives. To determine the cost per patient-month enrolled in an ART programme and per patient-visit for a dedicated, public-sector ART clinic in a South African peri-urban setting in 2004/05 and 2005/06, as the clinic moved from a temporary to a permanent site.

Methods. A retrospective costing study was performed from a programme perspective. Two years of expenditure data for the clinic were collected from primary sources. Costs per patient visit and per patient-month were calculated in Rand and converted to 2004 US$ (R6.4347 = US$1).

Results. The total cost of running the site, excluding patient-specific items (medicines and medical tests), was $174 072 in 2004/05 and $421 872 in 2005/06. Cost per patient-month fell from $40.29 to $36.47, a 9% decrease; cost per patient-visit fell from $54.79 to $41.62, a 24% decrease. In 2005/06, 68% of all expenditure was on medical and pharmacy staff (versus 62% in 2004/05), 23% was on the employment of peer adherence counsellors (versus 35%), and the remaining 9% was on capital costs and supplies (versus 3%).

Conclusions. The increase in scale of operation for the provision of ART at this clinic allowed economies of scale to be reaped. Staff costs, both medical and support, comprised the large majority of total clinic costs, such that the erection of a dedicated building for the clinic had little impact on the economic cost of care.

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Although the provision of antiretroviral therapy (ART) through the South African public health care sector has expanded rapidly since its inception in 2003, it has been estimated that less than one-quarter of those in need of antiretrovirals (ARVs) were on treatment by the end of 2005.[1] Furthermore, the numbers of people requiring ART will continue to rise for the foreseeable future, as those currently too well to need ART join those continuing in the programme.

The South African government is committed to providing ARVs to all who need them.[2] Given the size of the epidemic, and the limited resources available for public health care, it is crucial that the most efficient models of ART provision are utilised in order to achieve this goal. Unfortunately, there is little existing information on the cost and cost-effectiveness of different models of ART care. This is particularly true of those primary and community care-based models most relevant to lower- and middle-income countries with generalised epidemics.[3-5] Early cost analyses of ART provision, conducted in North America and Europe, focused largely on the cost of hospital, rather than clinic-based, care.[6] However, one recent study[7] of Swiss clinics compared hospital-based with general practice-based ART provision. The authors found that although hospital-based care was more expensive, this was almost entirely because of differences in ARV prescribing patterns and that non-medication costs did not differ significantly between the study arms.

Two costing studies of middle-income countries that do not have generalised epidemics have been performed, viz. in Thailand, looking at hospital-based clinics,[8] and in Mexico.[9] The latter study covered 11 major clinics nationwide, but the analysis abstracted away the variation due to differences in site of care provision via clinic fixed-effects. This made any consideration of cost differentials due to different models of care impossible.

Almost nothing is currently known about the overall cost of providing ARV services through the public health care sector in Africa. A study from KwaZulu-Natal found that running a voluntary counselling and testing (VCT) clinic cost between $161 and $53 per client in 2002/03, falling as the number of clients seen rose.[10] The only existing cost study of ART provision in South Africa of which the authors are aware, found that a dedicated, primary care ART clinic in Khayelitsha in the Western Cape cost R145.15 ($22.56) per visit in 2002/03, excluding patient-specific items such as medicines and laboratory tests.[11]

A more recent, predominantly qualitative, comparison of different models of ART care provision in the Western Cape[12] noted the significant variations in staff costs across clinics depending on whether they operated at hospitals or primary health care facilities, and on the mix of clinical staff.

This study aimed to provide information on the operating costs of a doctor- and adherence counsellor-intensive clinic based in the Western Cape. The objectives of the analysis were first to measure the cost components of a dedicated ART clinic

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in South Africa, and second to determine how the importance of these components varied as the clinic grew in size. It is hoped that this will contribute to a fuller understanding of the likely evolution of the cost of providing ART as the national treatment programme expands.

Methods

Site description

The site studied, the Hannan Crusaid Treatment Centre (HCTC), is a dedicated ART clinic based at the Gugulethu Day Hospital in Nyanga, a peri-urban settlement close to Cape Town. Operating since September 2002 it was initially a joint project between the Desmond Tutu HIV Centre (DTHC), which is a not-for-profit HIV research organisation; the UK-based charity Crusaid; and the provincial government of the Western Cape (PGWC). It has subsequently been fully integrated into the provincial ART roll-out programme. The HCTC acts as the primary care provider to all individuals enrolled at the centre, providing ART and non-ART-related care. The work of the HCTC has been described previously.[13]

This study was conducted retrospectively covering the 2 financial years 2004/05 and 2005/06, from March 2004 to February 2006. At the beginning of the observation period the clinic was run from a temporary structure with 70 m^2 of floor space, in the grounds of the Gugulethu Day Hospital. In March 2005 the clinic relocated to a purpose-built clinic, 369 m^2 in size, on the same site. A wide range of medical and non-medical equipment was purchased for this new clinic.

All medical staff are employed by the PGWC. In March 2004 the staff consisted of 1 principal medical officer (PMO) and 1 professional nurse (PN). Two years later this had expanded to include 3 PMOs, 2 senior medical officers, 2 PNs, a staff nurse, a principal pharmacist and 2 assistant pharmacists. A unit manager was also employed during some of the period in question.

The Sizophila adherence counsellor programme is a peer-counselling project that has been an integral part of the HCTC ART programme since its inception. Counsellors are employed from the local community and are all openly living with HIV. Each counsellor is responsible for up to 50 patients, providing pre-treatment counselling, group education on living on ART, home visits to monitor adherence and ongoing treatment support. This support is intensified if ART adherence declines. The project is co-ordinated by a nurse employed by the DTHC and during the period of study utilised a cellphone-based reporting system for relaying messages and reporting ART pill counts from home visits.

Data collection and analysis

The cost analysis was performed retrospectively from a programme perspective. The cost of the buildings and their contents were sourced from receipts for the relevant items provided by the DTHC and PGWC. Costs were apportioned across all years of useful lifetime: the temporary building was assumed to have a lifetime of 10 years, the purpose-built building 30, the fixtures and fittings 5, and electronic equipment 3. All costs were discounted at 3%, in line with international standards.[14]

The monthly cost of employing medical staff was taken from PGWC wage rates in February 2006. The costing of the Sizophila was complicated by the programme being managed from the DTHC by the nurse co-ordinator. The cost of running her office at the DTHC was included in proportion to her time spent on Sizophila. The cost of developing, implementing and running the cellphone-based reporting system was also included.

The quantity of medical supplies, such as needles, gloves and thermometers, and of cleaning products used in 2005/06 by the centre, was estimated by the nurse in charge of ordering such items. The cost of these items was calculated from the PGWC medical depot catalogue. A cost per visit for those supplies was calculated and that cost attributed to 2004/05 visits.

Stationery for patient records was provided by the DTHC. This cost was calculated from receipts for all items except paper forms, for which costs were based on the number of sheets of paper used per visit multiplied by the cost of the paper and of photocopying.

All costs were standardised to average 2004 prices using South Africa's consumer price index.[15] Costs were then converted into US dollars using the average exchange rate for 2004 at the rate of US$1 to R6.4347.[16] Total costs were calculated by summing all categories. The total number of visits for each financial year, both scheduled and unscheduled, was taken from clinic records. The total number of patient-months was calculated by measuring the number of days each patient who enrolled at the HCTC before 1 March 2006 spent in the programme in each financial year, and dividing the total by 28.

Results

The total cost of running the HCTC rose by 142% from $174 072 in 2004/05 to $421 872 in 2005/06 (Table I). The total number of patient visits made in the 2005/06 financial year was 10 137, an increase of 219% over 2004/05, although the total number of patient-months on treatment rose by only 167%, from 4 321 to 11 569.

The move from a temporary structure to a purpose-built building meant that the annual economic cost of the centre's building and equipment rose more than eightfold from $3 075 to $29 221, far faster than the rate of increase in patient numbers. However, even in 2005/06 physical assets accounted for only 6.2% of total costs.

Table I. Cost components at the Hannan Crusaid Treatment Centre (2004 US$)

	2004/05	2005/06
Physical assets		
Building	2 636	22 993
Fixtures and fittings	439	2 991
Electronic hardware	-	3 236
Medical staff		
Doctors' salaries	82 427	178 540
Nurses' salaries	22 903	41 196
Pharmacists' salaries	2 489	50 423
Office manager's salary	-	17 670
Sizophila Counselling Programme		
Management and administration	15 152	15 788
Counsellor salaries	36 447	72 102
Cellphone-based reporting system	9 115	9 412
Supplies and overheads		
Medical supplies	1 086	3 466
Stationery	998	2 763
Overheads	381	1 484
Total cost*	174 072	421 872
Number of patient-visits	3 177	10 137
Cost per visit	54.79	41.62
Number of patient-months on treatment	4 321	1 569
Cost per patient-month on treatment	40.29	36.47

*Figures do not sum precisely because of rounding.

In contrast, the cost of the counselling programme rose more slowly than patient growth, increasing by 60% between the 2 years. As a result, the proportion of clinic costs due to this programme fell from 34.9% in 2004/05 to 23.0% in 2005/06.

The largest cost component at the clinic was that of clinical and managerial staff, which rose from $287 829 to $693 785 over the period of observation. This accounted for 61.9% of all costs in 2004/05, rising to 68.2% in 2005/06. More than three-fifths (62.0%) of this cost in the more recent year was due to doctors' salaries, with the remainder being split between nurses (14.3%), pharmacists (17.5%) and a unit manager (6.1%).

The cost per patient visit fell by 24.0% between the 2 years of observation, from $54.79 to $41.62 (Fig. 1). Although the proportion of costs attributable to physical assets rose, it had little impact on the overall cost per patient-month. More noticeable was the fall in the proportion attributable to the adherence counsellor programme. The cost per patient-month changed little between 2004/05 and 2005/06, falling from $40.29 to $36.47 (Fig. 2).

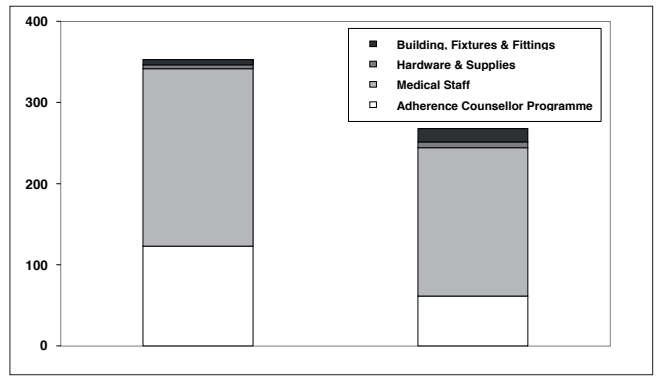

Fig. 1. Cost per patient visit (US$) at the Hannan Crusaid Treatment Centre.

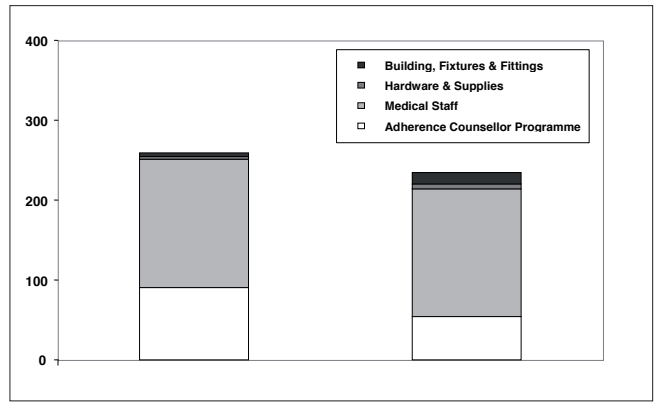

Fig. 2. Cost per patient-month (US$) at the Hannan Crusaid Treatment Centre.

Discussion

The reduction in cost per patient visit seen at this clinic over the period of observation, as services were rapidly scaled-up, suggests that there are significant potential returns to clinic scale in the provision of ARVs. Evidence that the clinic's clinical outcomes were not affected by this scale-up,[13] suggests that the reduction in cost per patient seen is not due to a reduced standard of care. The smaller reduction in cost per

patient-month can be attributed to the rise in the proportion of patients who were newly enrolled at the clinic, since clinic visits are more frequent in the first 6 months of care than thereafter.

The cost of running the HCTC was largely driven by staff salaries. This is in line with evidence from other South African studies that medical staff costs make up the majority of non-patient-specific expenditures at ARV clinics.[11] Unlike most other ARV clinics in South Africa, the HCTC is a heavily doctor-based programme,[12] which has the effect of raising the overall cost per visit well above that seen at another peri-urban clinic in the Western Cape.[11] The expansion in spending on staff salaries at the HCTC in this study period was proportional to the expansion in patient-months on treatment, but was slower than the rise in visits made, suggesting that some slight economies of scale may be achievable for medical staff.

An unusual aspect of the HCTC programme is the heavy use made of community-based adherence counsellors. The number of counsellors, even on a per-patient basis, is far higher than in comparable clinics across the Western Cape.[12] In March 2004 the counselling programme had been growing rapidly since its inception 18 months previously. It continued to expand, at a reduced rate, throughout the period of study.

Economies of scale in respect of training and administration were seen as the programme grew. This suggests that an intensive counselling intervention, such as the Sizophila model, is likely to be most efficient in large clinics, or when administration is shared between multiple sites.

The limitations of this study arise largely from the limited scope of enquiry made. No attempt was made to consider the effect of scale on demand for patient-specific items at the clinic, although it is likely that they remained approximately level on a per-visit basis. More importantly, no attempt was made to quantify what change, if any, was seen in the cost of care at other levels of care within the health care system; lower expenditure on primary care might have led to more secondary or tertiary care visits.

The HCTC ARV programme is a human resource-intensive one, employing more doctors and more counsellors per patient than other models of ARV care.[12] Nevertheless, significant economies of scale were reaped from expanding the programme between 2004 and 2006, particularly from the adherence counsellor component, without affecting clinical performance. Increased clinic scale appears to offer cost benefits in the South African ART roll-out.

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The authors declare no competing interests.

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The Evolving Cost of HIV in South Africa

Changes in Health Care Cost With Duration on Antiretroviral Therapy for Public Sector Patients

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Summary: A retrospective costing study of 212 patients enrolled in a nongovernmental organization–supported public sector antiretroviral treatment (ART) program near Cape Town, South Africa was performed from a health care system perspective. γ-Regression was used to analyze total costs in 3 periods: Pre-ART (median length = 30 days), first 48 weeks on ART (Year One), and 49 to 112 weeks on ART (Year Two). Average cost per patient Pre-ART was $404. Average cost per patient-year of observation was $2502 in Year One and $1372 in Year Two. The proportion of costs attributable to hospital care fell from 70% Pre-ART to 24% by Year Two; the proportion attributable to ART rose from 31% in Year One to 55% in Year Two. In multivariate analysis, Pre-ART and Year One costs were significantly lower for asymptomatic patients compared with those with AIDS. Costs were significantly higher for those who died Pre-ART or in Year One. In Year Two, only week 48 CD4 cell count and being male were significantly associated with lower costs. This analysis suggests that the total cost of treatment for patients on ART falls by almost half after 1 year, largely attributable to a reduction in hospital costs.

Key Words: cost, health care, highly active antiretroviral therapy, hospitalization, South Africa

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In nations with generalized HIV epidemics, the burden of HIV-positive individuals on the public health care system is considerable, often accounting for more than half of all inpatient care provided.[1,2] Provision of combination antiretroviral therapy (ART) in these settings is likely to have a significant impact on the annual, and particularly lifetime, cost of care, as it did when introduced to high-income countries.[3–5] Most comprehensive studies of health care costs to date have been conducted in North America and Europe.[6] Evidence from Africa is important, because the cost of providing ART to all who need it is likely to dwarf existing total health care expenditures on the continent; the cost of antiretroviral medicines alone is several times most African nations' current annual health care expenditures per capita.[5]

Given the variation in care delivery methods observed worldwide, it is not clear whether evidence from studies in non-African settings are comparable to those conducted on the continent. For example, antiretroviral medicines have typically comprised most of all treatment costs in high- and middle-income countries,[3,7–10] but existing South African studies have found them to contribute only a third of all costs.[11]

Evidence on the total cost of public health care for individuals on ART in Africa is limited.[11] In South Africa, one study calculated the program cost of running a clinic to provide ART to health care workers in KwaZulu-Natal to be between $1054 and $1382 per patient per year.[12] Three cost-effectiveness studies, 2 of hospital-based ART programs and 1 of a primary care ART clinic, also produced estimates of cost per patient-year.[13–15] All 3 showed that annual costs for those on ART were lower than for those not on ART, although lifetime costs were higher. In stratified analyses, those with low $CD4^+$ T-cell count or a history of AIDS had higher health care costs. One of these studies considered factors associated with hospitalization; however, in multivariate analysis, this study found neither CD4 cell count nor HIV RNA viral load level to predict use of services.[13] A costing study of the Western Cape provided a total cost per patient in a public sector clinic and total provincial expenditure requirements but not annual health care costs per patient.[16]

The only other study the authors are aware of that considered predictors of health care costs on ART outside a high-income setting was conducted in Mexico and found that patient health status measured by Centers for Disease Control and Prevention (CDC) stage, patient education level, and the specialty of the attending physician was associated with total cost.[10]

The first objective of this study was to determine the total annual cost to the health care system of a patient accessing a public sector ART clinic in South Africa according to time on ART, World Health Organization (WHO) stage, and CD4 cell count. The second objective was to analyze which of these factors were associated with significantly higher health care expenditures before treatment and in the first and second years on ART. It is hoped that this provides data for cost-effectiveness analyses and aids administrators in planning the provision of ART services.

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METHODS

Study Population

This study followed all eligible patients enrolled at the Hannan Crusaid Treatment Center (HCTC) between September 1, 2002 and December 31, 2003. The HCTC is a dedicated ART clinic based at the Gugulethu Day Hospital in Nyanga district, a periurban settlement close to Cape Town. During the period of study, it was jointly run by the Desmond Tutu HIV Center (a not-for-profit HIV research organization), the UK-based charity Crusaid, and the Provincial Government of the Western Cape (PGWC). Eligibility criteria for the clinic at the time of the study included a CD4 cell count of $<$200 cells/μL or a history of an AIDS-defining illness. The HCTC acts as the primary care provider to all individuals enrolled at the center, providing ART and non–ART-related care. Its work has previously been described.[17]

Patients are referred by local primary care providers. They are screened at the HCTC and seen a second time 14 days later, before commencing ART. Patients attend scheduled visits at weeks 0, 4, 8, and 16 after commencement and every 16 weeks thereafter. Patients may make unlimited unscheduled visits to the HCTC as required. First-line ART comprises stavudine, lamivudine, and efavirenz unless the patient is pregnant or a woman of child-bearing age not taking birth control medication; in such cases, zidovudine and nevirapine replace stavudine and efavirenz. The second-line regimen comprises lopinavir/ritonavir (Kaletra®), zidovudine, and didanosine. HIV RNA load, CD4 cell counts, and alanine transaminase tests are conducted before treatment and every 16 weeks during ART. Other safety tests are performed according to the provincial ART protocol and vary according to the treatment regimen.[18]

Subjects were followed from enrollment at the HCTC until their final scheduled 4-month visit in 2004, a maximum of 112 weeks after ART commencement. Patients were right-censored for death, loss to follow-up (LTFU), or transfer to another ART site. The baseline characteristics of this study population have previously been described.[19] Ethical approval for this study was gained from the University of Cape Town Research Ethics Committee, and all patients gave informed consent.

Data Collection

Costs were calculated from a public health care provider perspective. Data on patient-specific service utilization were collected prospectively from files at the HCTC and retrospectively from computer- and paper-based medical records at hospitals to which subjects were referred. The costs of antiretroviral medicines were taken from the 2004 national public sector tender, and those of other medicines were taken from provincial hospital tender prices. Medical test prices were taken from public sector tariffs charged by the National Health Laboratory Service and medical procedure costs from cost recovery charges made to private patients attending public hospitals.[20]

Non–patient-specific costs at the HCTC for the financial year 2005 to 2006 were calculated per patient-month in the program. Methods and results for this have been detailed previously.[21] Non–patient-specific costs for hospital visits were calculated from financial year 2004 to 2005 expenditure data, excluding all patient-specific line items,[22] on a per patient-day-equivalent basis using step-down accounting methods. Each inpatient day was weighted as 3.77 outpatient visits.[15] The cost of providing tuberculosis directly observed therapy (DOTS) was based on data collected in the same health district in 2000, calculated on a per-day-on-DOTS basis.[23] Unit costs for key inputs are shown in Table 1.

All costs were standardized to average 2004 prices using South Africa's consumer price index.[24] They were then converted into US dollars using the average exchange rate for 2004 of US $1 to R 6.4347.[25] Observation time was separated into 3 periods: between enrollment and ART commencement (Pre-ART), the first 48 weeks on ART (Year One), and any time after 48 weeks (Year Two). Costs were calculated per eligible patient enrolled in the program and per patient-year of observation (PYO; 365.26 days) in each period.

Multivariate Analysis

A multivariate regression model was used to determine which clinical factors were associated with demand for health care in each period. Utilization, and therefore cost, data are frequently noted to be right-skewed in distribution, with a large number of individuals consuming few resources and a few subjects using many. In this situation, ordinary least squares regression is not appropriate, because the data are not normally distributed and do not have constant variance.[26] To take these features into account, a γ-distribution with a log link was used.[27] The ability of this model to account for the nonnormal distribution was evaluated using deviance residuals.[28]

Explanatory variables used included age, gender, WHO stage at enrollment, CD4 cell count at the beginning of each period, change in CD4 cell count in the first 4 months on treatment, and whether the subject was censored for death or LTFU in the period. In all models, the dependent variable was

TABLE 1. Input Unit Costs ($US)

Antiretroviral regimens*	
Stavudine + lamivudine + efavirenz	31.88
Zidovudine + lamivudine + nevirapine	19.85
Zidovudine + didanosine + lopinavir/ritonavir	77.60
Monitoring and safety tests	
$CD4^+$ T-cell count	9.32
HIV RNA load	42.62
Alanine transaminase	4.76
Tuberculosis DOT†	71.84
Hospital inpatient day‡§	
Tertiary teaching hospital	393.92
Secondary district hospital	154.39
Dedicated tuberculosis hospital	55.09
Primary care clinic visit‡	75.53

*Cost of 28 days of treatment.
†Average 28-day period; costs varied by month and type of treatment.
‡Excludes patient-specific costs.
§Outpatient hospital visits were weighted at 26.5% of this cost.

the natural logarithm of costs. The primary outcome measure used was total cost per PYO. This is preferable to total cost per patient observed because it takes into account the intensity with which resources were consumed. The per-patient measure was used as a secondary outcome. Statistical analyses were performed using STATA 9.2 (Stata Corporation, College Station, TX). Differences between regression coefficients were tested using a joint Wald χ^2 test. All statistical tests were 2-sided at $\alpha = 0.05$.

RESULTS

Demographic and clinical characteristics of the sample are shown in Table 2. Just less than three quarters (72%) of the patients were female, and most (79%) were younger than 40 years of age. Most enrollees had late-stage HIV at program entry, with almost half (44%) having a previous AIDS-defining illness and more than half (58%) having a CD4 count of less than 100 cells/μL. After 16 weeks on the program, surviving patients had a median rise in CD4 count of 97 cells/μL, and after 48 weeks on the program, two thirds (66%) of patients had CD4 cell counts greater than 200 cells/μL. Most (78%) censoring events were attributable to death. For analytic purposes, all censoring events were assumed to be homogeneous and presented as a single group. Total length of follow-up was 25.2 patient-years in the Pre-ART period, 164.5 patient-years in Year One, and 88.6 patient-years in Year Two. During Year One, 5 patients moved from first-line to second-line therapy; during Year Two, a further 4 did so.

The mean number of hospital outpatient visits (excluding the HCTC) per patient in the cohort was 0.26 Pre-ART, 0.77 in Year One, and 1.05 in Year Two; the mean number of inpatient days was 3.04 Pre-ART, 3.73 in Year One, and 4.04 in Year Two. The mean number of hospital outpatient visits per PYO fell from 2.15 Pre-ART to 0.98 in Year One and 0.87 in Year Two; the mean number of inpatient days fell more sharply from 25.52 Pre-ART to 4.75 in Year One and 3.35 in Year Two.

The cost of providing treatment and care, to all patients and to those who were not censored, in each period is shown in Table 3. Total costs were $404 per patient between enrollment and ART commencement; the cost per patient for those who died in this period ($976) was more than twice this figure. Cost per PYO fell from $2502 in Year One to $1372 in Year Two. Censored patients in the Year One period cost an average of $16,645 per PYO, almost 5 times the average for that period. Per patient, this figure was $21,810. Censored patients in Year Two cost 4% less per PYO than the average for the period.

Figure 1 illustrates the division of costs in each period. In the Pre-ART period, one quarter (25%) of all health care costs were attributable to primary care visits to the HCTC, and almost all other costs (69%) were attributable to hospital-based care. In the Year One period, the cost of primary care and hospital care fell as a proportion of total health care costs, to 16% and 49%, respectively. The cost of providing ART amounted to 31% of total costs, with medicines accounting for 59% of these costs and monitoring tests for the remaining 41%. In the Year Two period, the absolute cost of ART fell slightly, with higher medicine costs being offset by lower monitoring test costs, but rose as a proportion of total health care costs to 55%. The proportion of costs attributable to primary care visits changed little but fell by 37% in absolute terms. The cost of hospital-based care fell rapidly, by 73% in absolute terms and by 50% in proportional terms. The cost of censored patients in the Pre-ART and Year One periods (not shown) comprised predominantly hospital care costs (88% and 85%, respectively), which were far higher in absolute and percentage terms than for other patients.

TABLE 2. Characteristics of Patients Included in the Analysis

	Pre-ART [n = 209]	Year One [n = 200]	Year Two [n = 132]
Days of follow-up, median (IQR)	30 (28 to 49)	336 (336 to 336)	224 (112 to 336)
WHO clinical stage			
1 or 2	22	21	16
3	94	93	60
4	93	86	56
CD4 cell count (cells/μL)	At baseline	At baseline	At 48 weeks
<100	122	116	4
101–200	71	68	41
201–350	16	16	60
>350	0	0	27
16-week CD4 cell count rise, median (IQR) [n = 181]		97 (49 to 153)	
Censoring events			
Death	10	20	2
LTFU		7	1
Transfer out		1	
Changes to second-line ART		5	4
Age, median (IQR) in years	33 (29 to 38)	33 (29 to 38)	33 (29 to 38)
Male	58	54	30

Figures are counts unless otherwise stated. One patient began ART without entering the Pre-ART period.

TABLE 3. Breakdown of Average Treatment Costs ($US) of Patients Enrolled in the HCTC Program by Time and Cost Component

	Pre-ART*		Year One†		Year Two†	
	Intention to Treat	On Program	Intention to Treat	On Program	Intention to Treat	On Program
No. patients	211	201	200	172	132	129
Antiretrovirals						
Medicine			454.41	457.53	488.85	490.65
Monitoring tests			320.26	309.29	269.79	269.65
Primary care clinic						
Overheads	8.60	8.55	28.15	26.82	17.61	17.66
Personnel	69.29	68.92	226.85	216.15	141.91	142.35
Medicines (not antiretroviral)	24.63	25.13	109.76	91.89	67.84	68.41
Tests and procedures	4.12	3.64	19.38	19.00	3.71	3.75
Adherence counseling	3.24	3.28	27.37	27.37	27.35	27.37
Hospital						
Overheads	107.47	95.19	447.96	277.32	98.04	97.86
Personnel	147.40	132.04	585.15	356.12	125.45	124.92
Medicines (not antiretroviral)	5.21	5.00	60.67	49.32	2.52	2.51
Tests and procedures	9.98	8.67	119.39	92.26	103.96	104.53
Primary tuberculosis treatment	24.00	25.20	102.19	96.69	25.15	25.40
Total cost per patient	403.93	375.62				
Total cost per PYO			2501.55	2019.77	1372.19	1375.06

*Costs are totals per patient.
†Costs are totals per PYO in the period.
Intention to treat includes all eligible patients beginning a period of observation; on program excludes those censored during the period.

Results of the multivariate regression analysis are presented in Table 4. In the Pre-ART period, patients in WHO stage 1 or 2 cost one fifth as much per PYO as those with an AIDS diagnosis, whereas those in WHO stage 3 cost 61% as much per PYO as those in stage 4 (see Table 4, upper panel). These differences were jointly significant. In contrast, a patient's baseline CD4 cell count, age, and gender were not significantly associated with health care costs. Costs per PYO for patients who were censored for death in the Pre-ART period were 7 times higher than for other patients. The odds ratio for censored patients dropped to 2.02 when the dependent variable was changed to cost per patient (see Table 4, lower panel), but all other coefficients remained roughly similar to those for cost per PYO.

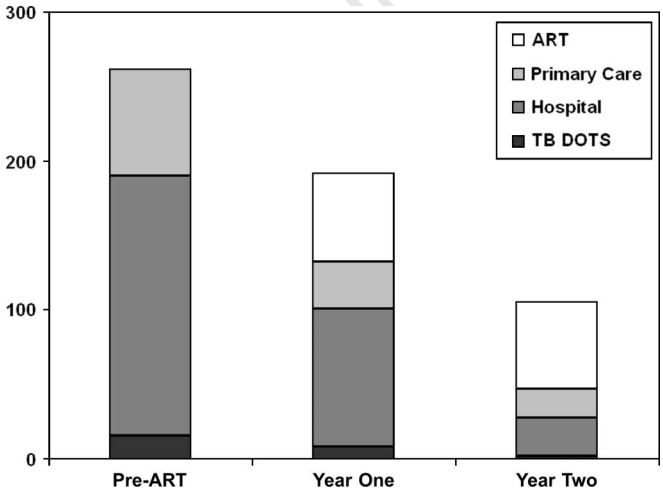

FIGURE 1. Monthly cost ($US) of care by time period. TB indicates tuberculosis.

In the first year on ART, significant predictors of health care costs continued to be WHO stage at program entry and censoring, although the odds ratios were closer to the null than in the Pre-ART period in both cases. The effect of a patient's baseline CD4 cell count continued to be nonsignificant. The change in CD4 cell count between baseline and week 16 on ART, however, was a significant predictor of costs, with each 50-cell/μL increase being associated with a 12% decrease in cost per PYO. Furthermore, men had a 25% lower cost per PYO after adjusting for all other variables, although this difference was not significant. Once again, the cost per patient analysis found similar results for all explanatory variables with the exception of censored patients, who nevertheless had significantly higher total costs than other members of the sample. The results for Year One shown in Table 4 do not include 19 individuals who did not have a week 16 CD4 cell count because of having been censored (15 deaths, 3 LTFU, and 1 transfer out). A regression containing all 200 observations and excluding week 16 CD4 cell count change as an explanatory variable (not shown) found similar results.

In the Year Two period, predictors of health care changed. Patients with a baseline WHO stage of 1, 2, or 3 still had lower

TABLE 4. Regressions of Health Care Costs for Patients Enrolled in an ART Program

	Pre-ART		Year One		Year Two	
Dependent variable: cost per PYO						
WHO stage 1 and 2	0.21	(0.07 to 0.63)	0.49	(0.24 to 1.00)	0.74	(0.50 to 1.09)
WHO stage 3	0.61	(0.30 to 1.20)	0.62	(0.39 to 0.97)	0.89	(0.69 to 1.15)
WHO stage 4	1.00	(reference)	1.00	(reference)	1.00	(reference)
CD4 count baseline	0.98	(0.74 to 1.28)	0.91	(0.77 to 1.08)		
CD4 count rise at week 16			0.88	(0.78 to 1.00)		
CD4 count week 48					0.95	(0.91 to 0.98)
Death, LTFU, transfer out	7.93	(1.90 to 33.0)	5.73	(2.05 to 16.0)	1.12	(0.49 to 2.53)
Age	0.98	(0.94 to 1.03)	0.99	(0.96 to 1.02)	1.01	(0.99 to 1.02)
Male	0.99	(0.46 to 2.13)	0.75	(0.45 to 1.25)	0.68	(0.51 to 0.91)
Joint χ^2 test (P value): all WHO stages = 1	7.95	(0.02)	5.89	(0.05)	2.47	(0.29)
Dependent variable: cost per patient						
WHO stage 1 and 2	0.17	(0.05 to 0.63)	0.49	(0.24 to 0.97)	0.61	(0.35 to 1.08)
WHO stage 3	0.59	(0.24 to 1.41)	0.60	(0.39 to 0.94)	0.77	(0.53 to 1.13)
WHO stage 4	1.00	(reference)	1.00	(reference)	1.00	(reference)
CD4 count baseline	0.90	(0.66 to 1.22)	0.91	(0.77 to 1.07)		
CD4 count rise at week 16			0.88	(0.79 to 1.00)		
CD4 count week 48					0.94	(0.89 to 0.99)
Death, LTFU, transfer out	2.02	(0.37 to 10.9)	2.77	(1.07 to 7.20)	0.36	(0.11 to 1.20)
Age	1.01	(0.96 to 1.06)	0.99	(0.96 to 1.02)	1.02	(0.99 to 1.04)
Male	0.88	(0.34 to 2.30)	0.72	(0.44 to 1.18)	0.73	(0.48 to 1.12)
Joint χ^2 test (P value): all WHO stages = 1	6.98	(0.03)	6.76	(0.03)	3.49	(0.17)
Observations (n)	209		181		132	

All figures are odds ratios (95% CIs), except where noted otherwise.
Odds ratios for CD4 counts are for 50-cell increments.

costs per PYO than those with an AIDS diagnosis but not significantly so. Furthermore, censored patients no longer had significantly higher costs per PYO. Instead, an individual's CD4 cell count at week 48 was a significant predictor, with each 50-cell/μL increment being associated with a 5% lower cost per PYO. Men cost 32% less per PYO than women.

Model checking using deviance residuals suggested that 1 patient, who had consumed a large proportion of all tertiary inpatient services, might have had a significant influence on the results in the Pre-ART and Year One periods. Dropping this observation from the regressions attenuated the coefficient on CD4 cell count rise in the Year One regression to 0.97 (95% confidence interval [CI]: 0.91 to 1.04) but did not significantly affect any other results.

DISCUSSION

The patients seen in this study were of similar age and clinical features as those seen in other early public sector ART cohorts in sub-Saharan Africa.[29–31] This analysis found that the average cost per patient-year for those enrolled in an ART program fell rapidly from the immediate Pre-ART period to the first 48 weeks on treatment and then fell by almost 50% over the following 64 weeks. It further found that predictors of cost shifted from WHO stage at program entry to CD4 cell count change in the first 16 weeks on treatment, and then current CD4 cell count at 48 weeks on ART.

The strengths of this study include the comprehensive data collection process, which included all persons enrolled at the clinic studied, and all care received by these patients at public health care facilities during the follow-up period. Because the clinical inclusion criteria at this facility were the same as those used in the national treatment program and the WHO's treatment criteria recommendations, it is likely that the health care cost patterns observed here are representative of those elsewhere in the country, and possibly Africa as a whole.

There were 2 important limitations to the study. First, the costing of the tertiary hospital included all costs at the institution, despite it being an academic institution. The inclusion of these nonclinical costs may have skewed the data. Concerns on this front are allayed by a sensitivity analysis where per diem personnel costs were reduced by 45% to reflect staff costs calculated in a microcosting study conducted in 1999/2000 that excluded academic components, adjusted for inflation to 2004.[32] This adjustment reduced the per diem cost of a visit to the tertiary hospital by 21%, but reduced the annualized cost of care by far less: 6.7% in the Pre-ART period, 6.7% in Year One, and 3.0% in Year Two.

Second, this study may be limited by the relatively small sample size. In particular, only 25 years of Pre-ART follow-up were available for analysis. The results may thus have been influenced by a few outlying large values, although the model specification reduced this concern in multivariate modeling. Nevertheless, the influence of a single observation was

sufficient to attenuate the result seen for CD4 cell change by 75%, and although there is no theoretic justification for this patient's exclusion from the sample, this finding reinforces the preliminary nature of these cost data.

Total average annualized costs in this population, particularly in the Pre-ART and Year One periods, are higher than those previously reported in the South African public sector, although they are lower than private sector program annual costs (Table 5). Part of this disparity may be attributable to this study's focus on patients initiating treatment rather than on total on-ART lifetime costs: costs were far lower for the Year Two period than for Year One. High pre- and early on-ART morbidity is likely to make direct comparisons between average lifetime costs and the Pre-ART and Year One costs in this study unreasonable, particularly given the short period of Pre-ART follow-up (median = 30 days).

The high average cost of care suggests that at least part of the gap is attributable to higher resource consumption in this population than in other public sector settings. Contributing factors may include the relatively high cost of the HCTC compared with other primary care clinics[21] or a differential pattern of service utilization. Utilization patterns may differ because of the availability of services or of variation in health need. The high cost of the HCTC reflects its high ratio of doctors to nurses relative to comparable public sector sites in the Western Cape.[36] This ratio arose early in the program's life, when it was one of only a handful of public sector ART clinics in country, and is now falling. The ratio may reflect the heavy use of privately funded adherence counselors, who perform many of the roles traditionally played by nursing staff in South Africa.[17] Given these observations, further data are needed to evaluate fully which level of costs is most representative of the South African public ART program.

One cost factor that has changed rapidly in recent years is the cost of antiretrovirals themselves. A recent review of published sources on ART costs in Africa found annual drug costs that ranged between $278 and $1540.[11] The levels reported in this study (range: $454–$491) are similar to those of other South African studies although higher than those seen elsewhere in Africa. In line with other studies from South Africa, these drug costs are lower in absolute and relative terms than those seen in higher income settings.

Predictive factors seen in the multivariate models are similar to those observed elsewhere and reflect predictors of mortality seen on ART. The importance of WHO stage has been noted in past cost models in various settings.[9,10,13,14] This study adds nuance to this result, however, finding that after 1 year on treatment, baseline WHO stage ceased to be predictive of such costs. This is in line with evidence that the importance of a historic AIDS-defining illness in predicting death decreases dramatically after 6 months on treatment and is not significant after 3 years.[37] After 12 months on ART, difference in CD4 cell count becomes the strongest clinical predictor of health care costs, as has been seen elsewhere.[7,9]

In addition to WHO stage, in the Year One period, the change in CD4 cell count by 16 weeks was a significant predictor of health care demand in this population. This reflects the importance of CD4 cell count response in the early months of treatment previously reported for mortality at this site.[38] The result in the current study, however, is independent of mortality, because death is adjusted for in the model. Also, those dying in the first 4 months on treatment are not included in the Year One analysis shown because they cannot have a week 16 CD4 cell count result. This suggests that the effect of low response to ART is to increase mortality and morbidity, although the fragility of this result to the inclusion of a single observation must make this finding a cautious one that would benefit from further investigation.

In the Year One and Year Two periods, being male was associated with reduced health care expenditures, significantly so by Year Two. An analysis of the differences in categories of cost (not shown) found the most important difference in resource use to be at the primary care level, where men made one third fewer unscheduled annual clinic visits than women on average. This difference was, however, not statistically significant.

Some effects seen in past studies were not observed in this setting. Although age is a traditional risk factor for general mortality and morbidity, such an association is not seen in this population. This is likely to reflect the narrow range of ages covered in this population, with 50% falling between 29 and 39 years old and only 4 individuals being more than 50 years old. In Mexico, variation in health care demand arose from differences in supply-side factors such as income and access

TABLE 5. Comparison of Estimates of Average Costs of ART Provision in South Africa

First Author	Treatment Setting	Population Treated	Cost/Patient/Year	Costs Excluded
Churchyard[33]	Private	Employees and dependents	$2287–$2761	Maybe inpatient and OI care
Cowlin[34]	Private	Insured individuals	$2173	Unclear
Badri[13]	Public hospital-based clinic	Clinical trial patients	$1513	
Deghaye[12]	Public hospital-based clinic	Health care professionals	$1031	Inpatient and OI care First-line ART only
Martinson[35]	Nongovernmental primary clinic	Public sector patients	$1322	Inpatient care
Cleary[15]	Nongovernmental primary clinic	Public sector patients	$1023	
Harling	Public primary clinic	Public sector patients	$2502 (Year One) $1372 (Year Two)	

Base year for costs, degree of discounting, and items included in costing vary.
OI indicates opportunistic infection.
Adapted from Rosen et al.[11]

to services.[10] This study is not able to speak to such matters, because the population seen here was economically and socially homogeneous, coming from a single district and having almost uniformly no earned income.

This study is among the first to analyze cost data for patients initiating ART in Africa. It shows a 31% decrease in cost per patient-year in the first year of ART provision compared with the immediate pretreatment period. It also shows another 45% fall in cost per patient-year between the first and second years of ART. This study suggests that factors predictive of costs change from clinical stage early in the treatment process to CD4 cell count after 12 months on treatment. These findings reinforce the importance of beginning ART before AIDS diagnosis in reducing the short-term burden of HIV on health care systems in countries with a high burden of HIV and limited resources to deal with it.

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MAJOR ARTICLE

Scaling Up Antiretroviral Therapy in South Africa: The Impact of Speed on Survival

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(See the editorial commentary by Hirschhorn and Skolnick, on pages 1223–5.)

Background. Only 33% of eligible human immunodeficiency virus (HIV)–infected patients in South Africa receive antiretroviral therapy (ART). We sought to estimate the impact of alternative ART scale-up scenarios on patient outcomes from 2007–2012.

Methods. Using a simulation model of HIV infection with South African data, we projected HIV-associated mortality with and without effective ART for an adult cohort in need of therapy (2007) and for adults who became eligible for treatment (2008–2012). We compared 5 scale-up scenarios: (1) zero growth, with a total of 100,000 new treatment slots; (2) constant growth, with 600,000; (3) moderate growth, with 2.1 million; (4) rapid growth, with 2.4 million); and (5) full capacity, with 3.2 million.

Results. Our projections showed that by 2011, the rapid growth scenario fully met the South African need for ART; by 2012, the moderate scenario met 97% of the need, but the zero and constant growth scenarios met only 28% and 52% of the need, respectively. The latter scenarios resulted in 364,000 and 831,000 people alive and on ART in 2012. From 2007 to 2012, cumulative deaths in South Africa ranged from 2.5 million under the zero growth scenario to 1.2 million under the rapid growth scenario.

Conclusions. Alternative ART scale-up scenarios in South Africa will lead to differences in the death rate that amount to more than 1.2 million deaths by 2012. More rapid scale-up remains critically important.

South Africa has one of the largest burdens of HIV disease in the world, with an estimated 4.9–6.1 million people infected and a reported prevalence of 18.8% in adults 15–49 years old [1]. In 2007, the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that 1,000,000 people in South Africa required antiretroviral therapy (ART) [2]. The number of patients with access to ART is steadily increasing, largely as a result of funding from the South African government itself, as well as strategic assistance from the US President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to

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Table 1. Alternative scale-up scenarios for antiretroviral therapy analyzed in the study.

Scale-up scenario	Description	New treatment slots available in year $_{(t+1)}$
Zero growth	There are no new treatment slots[a]	New slots$_{(t+1)}$ = 0
Constant growth	A fixed number of new slots open each year	New slots$_{(t+1)}$ = new slots$_{(t)}$
Moderate growth	Each year, 100,000 additional new slots open, compared with the prior year	New slots$_{(t+1)}$ = new slots$_{(t)}$ + 100,000
Rapid growth	Each year, the number of new slots doubles, compared with the prior year	New slots$_{(t+1)}$ = min ([2 × new slots$_{(t)}$], patients in need$_{(t+1)}$)[b]
Full capacity	Each year, there are slots available to treat everyone in need	New slots$_{(t+1)}$ = Patients in need$_{(t+1)}$ − existing slots freed up by deaths$_{(t)}$

[a] This reflects newly available treatment slots for antiretroviral therapy (ART) and does not reflect treatment slots that became available due to deaths of patients who received ART in the previous year.
[b] The no. of new slots is equal to whichever of these 2 values is smaller.

Fight AIDS, Tuberculosis and Malaria (GFATM) [3–5]. Treatment efforts have been accompanied by a national AIDS plan for drug distribution, creation of a more comprehensive health care infrastructure, and establishment of treatment guidelines [6]. Even with these remarkable expansions in capacity, however, the enormity of the epidemic continues to overwhelm the treatment systems; by the end of 2006, ART was reaching fewer than 33% of eligible patients in South Africa [7].

For development of health policy, it is useful to quantify the potential consequences of disease outcomes under different treatment and practice scenarios [8]. To this end, our objective was to project alternative ART rollout scenarios over the next several years in South Africa. The goals were to forecast the number of lives lost while awaiting needed therapy, to estimate the number of people both in and out of care, and to project when and whether HIV treatment needs would be fully met. These estimates can be used to inform decisions regarding the life-saving value of alternative treatment expansion scenarios in South Africa, as well as in other developing countries.

METHODS

Analytic Framework

We conduct this analysis in 4 steps. First, we parameterized a detailed computer-based simulation model of HIV disease with data from South Africa. Second, we conducted a series of analyses that assessed the outcomes associated with different ART strategies in selected cohorts of patients. Outcomes included mean CD4 count and HIV RNA levels of the surviving cohort over time, as well as AIDS-related and non–AIDS-related deaths each year. Treatment strategies included no ART and ART that used 2 lines of therapy ("line" here refers to the modeled number of sequential ART regimens available). Each strategy included co-trimoxazole prophylaxis in all scenarios, provided according to WHO guidelines [9]. Cohorts were selected to reflect the fact that in 2007 (the year of the most up-to-date WHO estimates) [2], patients who met ART eligibility criteria were distributed across HIV disease stages, as well as the fact that in subsequent years, patients who were initially in earlier disease stages progress to meet ART criteria. Third, we used the information generated from the model simulations to assess outcomes associated with different scenarios intended to represent potential population-based strategies for ART scale-up (table 1). Outcomes were expressed as number of people alive and on ART, as well as deaths, between 2007–2012. Finally, in sensitivity analyses, we examined the effect of changes in the CD4 count distribution of the cohorts modeled, the number and efficacy of available ART regimens [10, 11], and the availability of CD4 count monitoring [12].

CEPAC-International Model and Parameterization

The model. The CEPAC-International Model is a computer-based simulation model of the natural history of HIV infection in different settings; it has been used to estimate the clinical and economic consequences of different strategies for opportunistic infection prophylaxis and ART [13, 14]. We provide a focused description here; further model details have been described elsewhere (see the Appendix, which is available only in the electronic version) [13, 14]. Disease progression was portrayed as a sequence of monthly transitions between health states defined to capture key elements of disease and prognosis. Health states reflected chronic HIV infection, acute illness related to HIV (e.g., opportunistic infection), or death. Health states were stratified by HIV RNA level (>30,000 copies/mL; 10,001–30,000 copies/mL; 3,001–10,000 copies/mL; 501–3,000 copies/mL and ⩽500 copies/mL), which informs the monthly rate of decline in CD4 count. In turn, CD4 count (⩽50 cells/μL, 51–200 cells/μL, 201–350 cells/μL, 351–500 cells/μL, and >500 cells/μL) informs the monthly risk of opportunistic infection and death (table 2). Clinical decisions, such as the initiation of ART, were based on clinical findings and, when available, CD4 count and HIV RNA results. Patients on effective ART experience monthly increases in CD4 count, reducing their risk of opportunistic infections and death. After virologic failure, CD4 counts decline, with a concomitant increase in the risk of opportunistic infections. Patients who remain on ART despite virologic failure continue to have some reduction in risk of opportunistic infections and mortality

Table 2. Model input parameters for a simulation of scale-up of antiretroviral therapy (ART) in South Africa.

Variable	Estimate	Reference
Monthly decline in CD4 count, mean, cells/μL		[15]
>30,000 HIV RNA, copies/mL	6.4	
10,001–30,000 HIV RNA, copies/mL	5.4	
3,001–10,000 HIV RNA, copies/mL	4.6	
501–3000 HIV RNA, copies/mL	3.7	
≤500 HIV RNA, copies/mL	3.0	
Monthly risk of severe opportunistic infections[a], %		[16]
Bacterial	0.08–0.71	
Fungal	0.02–2.22	
Tuberculosis	0.21–1.96	
Toxoplasmosis	0.00–0.06	
Non-tuberculous mycobacteriosis	0.00–0.30	
Other	0.25–2.57	
Monthly risk of mild opportunistic infections[a], %		[16]
Pneumocystis jiroveci pneumonia	0.00–0.12	
Fungal	0.59–3.51	
Other	2.51–3.11	
Efficacy of co-trimoxazole, percent reduction in monthly risk of infection		
Mild bacterial infection	48.8	[14]
Severe bacterial infection	49.8	
Severe malaria	88.4	
Isosporiasis	81.8	
Toxoplasmic encephalitis	83.3	
Mild fungal infections[b]	−46.4	
Rates of virologic suppression at 48 weeks, %		
NNRTI + 2 NRTI	84	[17, 18]
PI + 2 NRTI (recycled NRTIs)	71	[19]
2007 prevalent cohort		Model derived
CD4 counts, cells/μL, mean ± SD	92 ± 49	
Initial distribution of viral load, percentage of patients		
>30,000 HIV RNA copies/mL	67	
10,001–30,000 HIV RNA copies/mL	19	
3,001–10,000 HIV RNA copies/mL	9	
501–3,000 HIV RNA copies/mL	3	
≤500 HIV RNA copies/mL	2	
2008–2012 incident cohorts		Model derived
CD4 count, mean ± SD, cells/μL	210 ± 50	
Initial distribution of viral load, percentage of patients		
>30,000 HIV RNA copies/mL	71	
10,001–30,000 HIV RNA copies/mL	18	
3,001–10,000 HIV RNA copies/mL	8	
501–3,000 HIV RNA copies/mL	2	
≤500 HIV RNA copies/mL	1	

NOTE. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a] Risk is stratified by CD4 cell count; in nearly all cases, monthly risk increased with decreasing CD4 count. The following 5 severe opportunistic infections were considered late stage and were used to trigger initiation of antiretroviral therapy: severe fungal infections, isosporiasis, cerebral toxoplasmosis, non-tuberculous mycobacteriosis, and other severe illnesses.

[b] The percentage monthly risk of mild fungal infections is increased by 46.4% in the presence of co-trimoxazole [14].

independent of CD4 count [20], compared with patients who do not continue therapy. The model specified the most prevalent opportunistic infections in South Africa and divided them into 8 severe and 3 mild categories (table 2).

Model input parameters. Model input data are summarized in table 2 [14–19, 22]. Model results for both the treated and untreated cohorts were validated with current HIV-related death rates in South Africa [21, 22]. Data on the monthly risk of opportunistic infection were derived from a Cape Town cohort of 2,080 patients followed up longitudinally since 1984 [16, 23–25]. They were stratified by CD4 count, and the highest risk of opportunistic infection occurred in the lower strata [16, 23–25]. Co-trimoxazole is effective in preventing several of these opportunistic infections (e.g., *Pneumocystis jiroveci* pneumonia [formerly *Pneumocystis carinii* pneumonia], toxoplasmosis, and some bacterial infections), though it may also increase the risk of mild fungal infections [14].

A first-line, nonnucleoside reverse transcriptase inhibitor–based regimen (in combination with 2 nucleoside reverse transcriptase inhibitors [NRTIs]) had a 48-week HIV RNA suppression rate of 84%, with a mean CD4 count increase of 184 cells/μL [17, 18]. Upon observed clinical or immunologic failure of the first-line regimen, the efficacy of a second-line regimen, if available, presumed the addition of a ritonavir-boosted protease inhibitor. This second-line regimen provided a 48-week HIV RNA suppression rate of 71% and a corresponding CD4 count increase of 151 cells/μL [19].

Cohort Descriptions

We distinguished patients who required therapy in 2007 (hereafter, the "prevalent cohort") from those who would need therapy in the period from 2008–2012 (hereafter, the "incident cohort"). The outcomes of both cohorts were examined for both the ART and "no ART" strategies. We defined patients in need of ART as those who met the current South African criteria that define ART eligibility, regardless of whether the patient had been identified as having HIV infection [26, 27]. These criteria include WHO Stage 4 AIDS-defining illness and/or symptomatic disease with a CD4 count $<$200 cells/μL [26, 27]. In the base case, there is no prioritization for ART; that is, among those eligible, patients are randomly chosen for ART and are not given preference based on CD4 count, degree of "AIDS sickness," or time waiting for ART.

Patients in need of therapy in 2007. We began the time horizon with the most recent WHO estimates (2007), when there were an estimated 1,000,000 patients in South Africa in need of therapy [2]. To approximate the distribution of stage of illness for these patients, we assumed that the South Africa epidemic was in a steady state and that the number of incident cases each year remained the same over the 10-year period prior to ART rollout (1997–2006). As noted above, we refer to this cohort as the prevalent cohort.

Patients who require therapy in the future (2008–2012). Patients become newly eligible for therapy each year from 2008–2012. This reflects the fact that incident cases of HIV will occur, and that existing patients with early infection will progress to a point at which they are eligible for ART. Approximately 5.51 million people are estimated to be living with HIV/AIDS in South Africa [2, 28]. With a mean 10-year progression from HIV infection to AIDS [29], we estimated that 10% of those 5.51 million infected people (or 551,000 people) would be newly in need of therapy in each year. As noted above, we refer to this group as the incident cohort.

Derivation of initial mean CD4 counts of the cohorts. The mean CD4 count of the prevalent and incident cohorts were obtained from the CEPAC-International Model. The incident cohort was derived first. The characteristics of the incident cohort, which becomes eligible for ART in yearly cycles, are obtained by tracking the course of HIV disease for a relatively healthy starting cohort (CD4 count, mean $\pm$ SD, 534 $\pm$ 100 cells/μL) that received only co-trimoxazole prophylaxis [30]. The model records and averages the clinical characteristics of patients (e.g., CD4 count, HIV RNA levels, and opportunistic infection history) as they reach the criteria for ART eligibility (CD4 $<$ 200 cells/μL or severe opportunistic infection). The resulting incident cohort had a mean ($\pm$SD) CD4 count of 210 ($\pm$ 50) cells/μL.

The prevalent cohort was a heterogeneous distribution of patients who had been in need of ART for varying amounts of time. The characteristics of the prevalent cohort were obtained by simulating and tracking the course of HIV disease for an incident cohort as described above (CD4 count, mean $\pm$ SD, 210 $\pm$ 50 cells/μL) that receives co-trimoxazole prophylaxis only [14]. Thus, the 1997 cohort was followed for 10 years, whereas the 2006 cohort was followed for 1 year. We then calculated a weighted average of CD4 count and HIV RNA distribution on the basis of the population distribution of all patients who were alive at the end of the 10-year period. The patients alive at the beginning of the ART rollout period consisted mostly of the cohort that had become eligible in 2006 (44% of the patients alive at beginning of the rollout period), whereas patients who had reached the eligibility criteria in 1997 but were not treated constituted less than 0.01% of those still alive in 2007. The resulting cohort (i.e., the prevalent cohort) had a mean ($\pm$SD) CD4 count of 92 ($\pm$ 49) cells/μL.

Scenarios Examined

We examined 5 population-based strategies of ART scale-up based, in part, on projections made by the Actuarial Society of South Africa, the South African Joint Task Force, and the WHO [6, 28, 31]. These scenarios (table 1) range from a situation in which no new treatment funds are allocated (zero growth) to a situation in which a rapid and aggressive scale-up program is initiated (rapid growth) [32, 33]. We consider the full capacity scale-up program only for purposes of comparison.

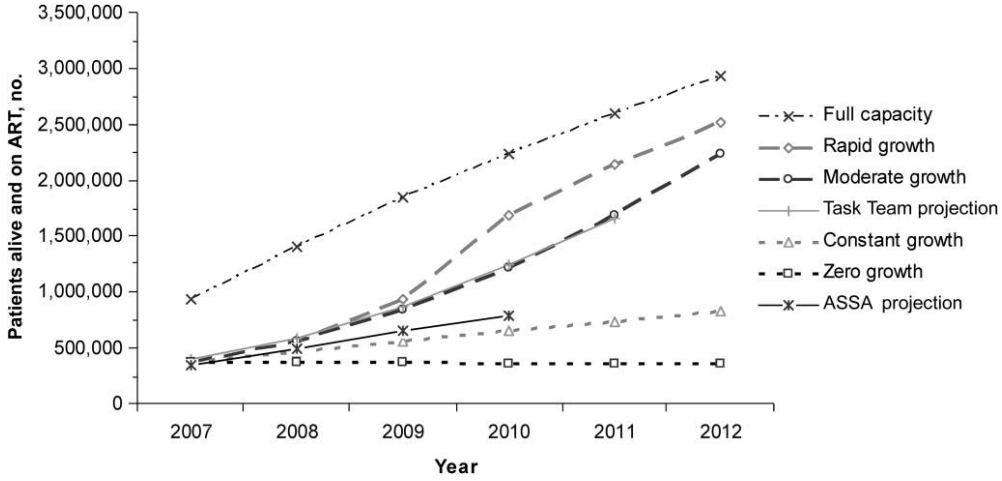

Figure 1. No. of patients alive and on antiretroviral therapy (ART) at the end of each year, according to scale-up scenario *(dashed* and *dotted lines),* compared with previously published South African projections *(solid lines).* The constant growth scenario modeled in the study best approximates the projections of the Actuarial Society of South Africa (ASSA), whereas the moderate growth scenario best approximates the projections of the South African Joint Task Team on HIV Care in the Public Sector [6, 28, 31].

Using WHO surveillance data and model-generated mortality rates, we projected the number of deaths under each scenario. Each year, mortality was calculated to include deaths that occurred among patients who needed, but had not yet received, ART, in addition to deaths that occurred—albeit later and at a slower rate—among patients who received ART. For patients who died while receiving ART, we assumed that their treatment slots (which reflected unused medications and human resources) would be available for other patients in the following year. In each scenario, the total deaths and total life years of survival were tallied and reported from 2007–2012.

RESULTS

Survival Rates

Assessment of model performance. To ensure that model-based death rates were appropriate for long-term projections, we compared them to reported survival trends in South Africa, both for patients who received ART and those who did not. At each yearly time point, model-based estimates were within 10% of reported survival (see the Appendix, which is available only in the electronic version) [21].

Projected survival with and without ART for both cohorts. Patients who entered the model in the prevalent cohort (2007; mean CD4 count, 92 cells/μL) and received ART in that year had 1- and 5-year projected survival rates of 94% and 70%, respectively. If they did not receive ART, these patients had markedly reduced survival rates of 55% at 1 year and 1% at 5 years. For patients in the comparatively healthier incident cohort (2008–2012, mean CD4 count, 210 cells/μL) who received ART as recommended without delay, 1- and 5-year survival rates were 94% and 72%, respectively. If the patients did not receive ART, the corresponding 1- and 5-year survival rates for the incident cohort were 79% and 4%.

Number of Patients Who Received ART for Each Scenario

For each scale-up scenario, we examined the number of people alive and on ART at the end of each year, comparing the current study to published South African projections (figure 1). By 2012, if all patients received ART in the year that they first met eligibility criteria (full capacity), 2.9 million people would be alive and receiving treatment in 2012. The rapid-growth scenario results in 2.5 million people receiving treatment, compared to 2.2 million in the moderate-growth scenario, 831,000 in the constant-growth scenario, and 364,000 in the zero-growth scenario.

Figure 2 illustrates the percentage of patients alive and eligible each year who received ART. All of the patients who needed ART had access by 2011 in the rapid-growth scenario; the moderate-growth scenario met 97% of treatment need by 2012. In 2012, the constant-growth scenario met only 52% of the treatment need; the zero-growth scenario—with no new treatment slots available after 2007—met just 28% of the treatment need by 2012 (figure 2).

Mortality Projections for Each Scenario

The number of deaths projected to occur before patients started ART and while patients were receiving ART were also calculated for each of the scenarios (figure 3). The total number of deaths increases over time in the zero-growth scenario due to a yearly influx of patients who require treatment (the incident cohort) without the provision of any new treatment resources. The rapid-growth scenario results in notably fewer deaths than all the other scenarios (except full capacity, which is provided for purposes of comparison). The increase in deaths under the rapid-growth scenario in 2010–2012 reflects patients who received

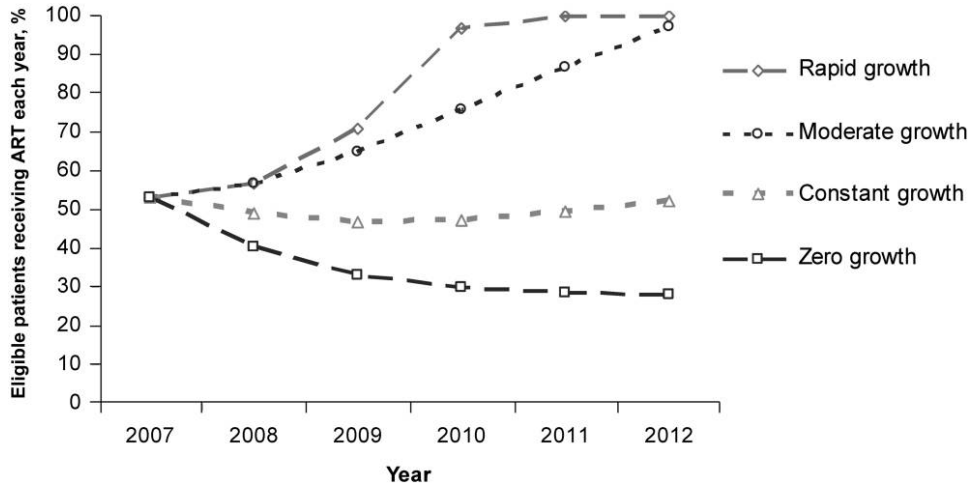

Figure 2. Percentage of patients eligible and alive who received antiretroviral therapy, for each modeled scenario. The rapid-growth scenario met 100% of need by 2011, and the moderate-growth scenario met 97% of need by 2012. The constant-growth scenario remained relatively unchanged and met 52% of need by 2012. The percentage of need met in the zero-growth scenario declined steadily to 28% in 2012.

ART during earlier years of their illness but who subsequently failed ART, and who die several years after treatment initiation.

Table 3 summarizes outcomes associated with the nearly 3.8 million patients eligible for therapy in the time horizon considered. The total number of deaths over 6 years ranged from 2.5 million under the zero-growth scenario to 1.2 million under the rapid-growth scenario. Compared with the zero-growth scenario, the constant-growth and moderate-growth scenarios averted 305,000 and 1,000,000 more deaths through 2012, respectively.

In a sensitivity analysis, we examined the assumption that patients in need of ART are prioritized on the basis of the length of time they have been eligible for therapy (table 3). For example, in this prioritized scenario, the whole 2007 prevalent cohort would be treated before anyone in the 2008 incident cohort received therapy. This sort of prioritization, on average, treats the sickest patients (i.e., those with the lowest CD4 counts) first. For each scenario, prioritization led to fewer deaths over the 6-year time horizon, compared with the base case analysis. Comparison of each scenario with its nonprioritized counterpart showed between 17,000 (zero-growth scenario) and 87,000 (moderate-growth scenario) fewer deaths over 6 years.

We also examined the availability of only 1 line of ART (rather than 2 successive lines), changes in the efficacy of second-line therapy, the availability of 3 successive lines of ART, and a sce-

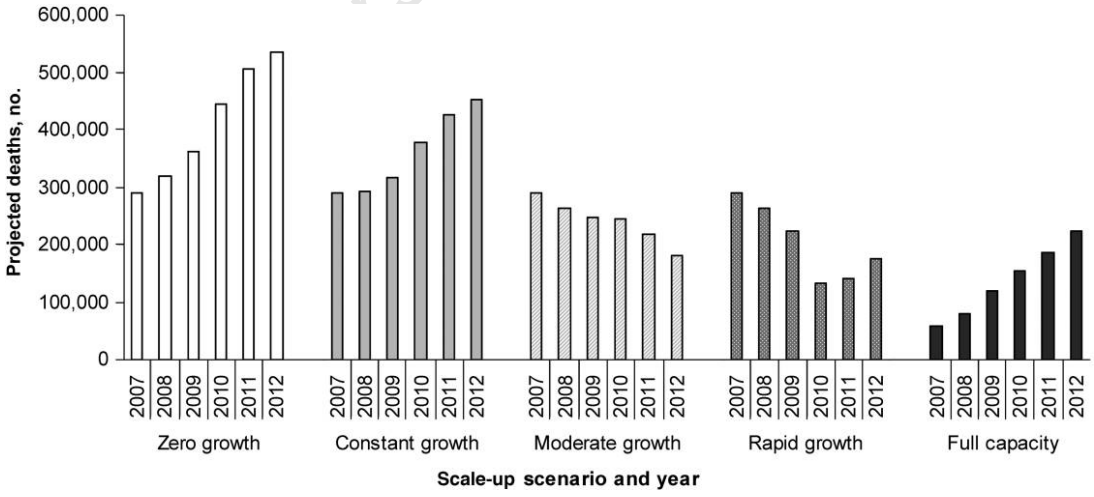

Figure 3. Annual projected no. of deaths through 2012, stratified by scale-up scenario and year. All scale-up scenarios (except full capacity) involved the same number of treatment slots in 2007, and all scenarios included the availability of 2 successive lines of therapy. The estimated number of deaths each year for those awaiting antiretroviral therapy (ART) and those receiving ART were calculated for each scale-up scenario by use of mortality rates generated from model output. Annual mortality rates for both the prevalent and incident cohorts were stratified by treatment status (awaiting ART or receiving ART) and length of time awaiting therapy.

Table 3. Outcomes for patients eligible for antiretroviral therapy (ART), according to alternative scale-up scenarios for ART in South Africa, 2007–2012.

Analysis, scenario	Dead, no. of patients			Alive, no. of patients		
	Awaiting ART	On ART	Total	Awaiting ART	On ART	Total
Base case						
Zero growth	2,294,000	171,000	2,465,000	926,000	364,000	1,290,000
Constant growth	1,896,000	264,000	2,160,000	764,000	831,000	1,595,000
Moderate growth	978,000	471,000	1,449,000	64,000	2,242,000	2,306,000
Rapid growth	681,000	551,000	1,232,000	0	2,523,000	2,523,000
Full capacity	0	823,000	823,000	0	2,932,000	2,932,000
Sensitivity analyses						
Prioritization						
Zero growth	2,268,000	180,000	2,448,000	946,000	361,000	1,307,000
Constant growth	1,800,000	286,000	2,086,000	845,000	824,000	1,669,000
Moderate growth	888,000	474,000	1,362,000	152,000	2,241,000	2,393,000
Rapid growth	610,000	564,000	1,174,000	0	2,581,000	2,581,000
Full capacity	0	823,000	823,000	0	2,932,000	2,932,000
1 line of ART						
Zero growth	2,264,000	229,000	2,493,000	910,000	352,000	1,262,000
Constant growth	1,861,000	344,000	2,205,000	740,000	810,000	1,550,000
Moderate growth	943,000	590,000	1,533,000	23,000	2,200,000	2,222,000
Rapid growth	672,000	681,000	1,353,000	0	2,402,000	2,402,000
Full capacity	0	1,024,000	1,024,000	0	2,731,000	2,731,000
3 sequential ART regimens						
Zero growth	2,297,000	162,000	2,459,000	929,000	367,000	1,296,000
Constant growth	1,899,000	253,000	2,152,000	767,000	836,000	1,603,000
Moderate growth	981,000	458,000	1,439,000	68,000	2,248,000	2,316,000
Rapid growth	681,000	537,000	1,219,000	0	2,536,000	2,536,000
Full capacity	0	796,000	796,000	0	2,959,000	2,959,000
No CD4 monitoring						
Zero growth	2,027,000	541,000	2,568,000	862,000	325,000	1,187,000
Constant growth	1,539,000	897,000	2,436,000	611,000	708,000	1,319,000
Moderate growth	673,000	1,565,000	2,237,000	0	1,518,000	1,518,000
Rapid growth	566,000	1,652,000	2,218,000	0	1,537,000	1,537,000
Full capacity	0	2,101,000	2,101,000	0	1,654,000	1,654,000

nario in which no CD4 count monitoring was available. Compared with scenarios in which there were no CD4 tests available, the availability of CD4 testing resulted in 102,000–986,000 fewer deaths over 6 years. For each scenario, more deaths occurred when there were 2 lines of therapy and no CD4 monitoring available, compared with only 1 line of therapy and CD4 monitoring. Finally, varying the assumption regarding the efficacy of ART in preventing severe opportunistic infections, independent of CD4 count, produced quantitative changes in the survival rate but had no material impact on the qualitative findings.

DISCUSSION

We used a model-based analysis and South African data to project the average impact of alternative ART strategies over the next 5 years. We applied these strategies to different cohorts, which were intended to be representative of patients who were eligible for ART in 2007, patients who will be eligible for ART from 2008–2012, and patients who have been waiting for ART for varying periods of time. We aggregated the results of these cohorts to create a comparative analysis of population-based scenarios for ART scale-up.

The findings suggest that the potential loss of life associated with the failure to provide ART to all who need it, in South Africa alone, is enormous. A scenario that maintains current treatment capacity with no addition of treatment resources over the long term (zero growth) will result in over 1.2 million more deaths in South Africa—a country with a population of 48 million people [34]—by 2012, compared with a scenario that provides the resources to ensure universal access to ART by 2011 (rapid growth). The current projected time line for ART scale-up in South Africa (2.1 million receiving treatment by 2012, or a moderate-growth scenario) will likely result in nearly 1.5 million deaths through 2012, over 200,000 more than would be seen with rapid growth [35].

ART guidelines are generally country-specific and vary widely [36]. Some countries, for example, Malawi, do not recommend CD4 count monitoring, and ART initiation is based on WHO stage-3 or stage-4 disease, or CD4 count, if available [37]. Patients are now being enrolled in trials to examine the long-term impact of clinical monitoring versus laboratory monitoring while on ART, because earlier reports from programs that used clinical guidelines alone suggest substantial survival benefits, even without CD4 count monitoring [12]. These reports are consistent with the results reported here, however, without access to CD4 monitoring, the number of deaths through 2012 may increase by as many as 986,000, compared with similar scale-up scenarios in which CD4 monitoring is available. Indeed, these results emphasize that the addition of any ART regimen will greatly improve survival; however, they also highlight that the benefits of ART will be maximized with the addition of CD4 counts, as is current South Africa policy.

This analysis has several limitations. It does not compare different strategies for the operational delivery of ART (an essential component to effective scale-up), nor does it include unintended negative consequences of ART. Importantly, we also do not discuss the opportunity costs of investment in expanded ART. One South African projection estimates that full HIV treatment coverage by 2010—including the costs of antiretroviral drugs, nutritional support, and non-antiretroviral drugs—will cost 16.9–21.4 billion Rand in South Africa (US$2.54–3.22 billion) [38]. Although we have not addressed societal trade-offs in this paper, this analysis offers a quantitative assessment of the opportunity cost—in lives lost—of failing to make these investments in HIV care.

Beyond economic requirements, other complicated infrastructure issues cannot be overlooked and must play a role in any ART implementation. These include facilities, personnel, equipment, and political will. A goal of rapid scale-up must also be accompanied by a commitment to adequate monitoring of therapy, to avoid depletions of stock and interruptions in drug delivery, and to ensure patient adherence so that drug resistance does not occur. Recent findings on the role of extensively drug-resistant tuberculosis (XDR-TB) suggest that the implementation of a large-scale ART program in South Africa will require improvements in tuberculosis control programs as well, to maximize the survival benefits conferred by ART [39]. The rising prevalence of drug-resistant strains of tuberculosis, coupled with the high mortality rate among HIV-infected patients with XDR-TB, risks considerably undermining the benefits of ART in South Africa [39]. Infrastructure improvements for the care of individuals with HIV infection and tuberculosis are particularly important, given the possibility that XDR-TB may be transmitted in healthcare settings, thus putting HIV patients who seek medical care at greater risk of infection with this virulent tuberculosis strain [39].

As with all model-based analyses, data were derived from multiple sources, which contributes to some uncertainty in the model projections. In most cases, data are specifically from South Africa. However, when South African data were unavailable, we used the closest appropriate surrogate data. To ensure that the model outcomes adequately reflect the South African context, we provided validation from the South African natural history and treatment literature. The estimates of mean CD4 count in the population were made under the assumption of an HIV epidemic in steady state. We have addressed this assumption via sensitivity analyses, examining the parameters that might be most influential in the overall results. The analysis of prioritization made ART available first to those who had been waiting longest. On average, this meant prioritizing those with the lowest CD4 count (mean CD4 count when receiving ART after a wait of 1 year was 139 cells/μL, compared with a CD4 count of 210 cells/μL for those who received ART immediately), though this might not be true in every case.

Though the goal is ambitious, ART scale-up efforts to date have been impressive. The number of people receiving ART in low-income and middle-income countries worldwide increased more than five fold between 2001 and 2005, from 240,000 to 1.3 million [1]. This level of achievement was previously thought to be impossible. Deliberate, purposeful, and expedient scale-up—together with careful analysis of the outcomes associated with different programmatic approaches—will make the difference in saving hundreds of thousands, or millions, of lives.

CEPAC-INTERNATIONAL INVESTIGATORS

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APPENDIX

This appendix is intended to provide methodological details to supplement the article's description of methods. We also include here some additional model output and results.

METHODS

Defining the Cohorts for the CEPAC-International Simulations

We defined several cohorts for the model simulations. The first were the validation cohorts (treated and untreated), which were defined to compare output from the model to endpoints and outcomes reported in the literature. Next, we defined a prevalent cohort, intended to model the distribution of patients who needed therapy in 2007. Finally, we defined an incident cohort to represent the distribution of newly-eligible patients who will require therapy in the future (2008–2012).

Validation Cohorts

The validation cohorts were intended to reflect a previously published cohort and are used to illustrate the fact that model outcomes and survival are representative of findings that have been reported in the South African setting [21, 22]. As noted in the literature, the untreated cohort has a mean (±SD) CD4 count of 273 (± 231) cells/μL, and the treated cohort has a mean (±SD) CD4 count of 259 (± 153) cells/μL (table A1) [21]. These mean CD4 counts were used as model input parameters to assess whether model output reflected the survival rates seen in the reported cohorts. Badri et al. [21] report that 50% of patients who received co-trimoxazole alone (reflective of the untreated cohort) survived for 30 months, and 90% of patients who received ART were alive at 36 months. Model-based estimates report median survival times of 31.5 months on co-trimoxazole prophylaxis alone, and 82.3% of patients who received ART were alive at 36 months. As noted in the Results section, model-based estimates reflected reported survival within 10%.

Table A1. Validation cohort characteristics.

Variable	Estimate	Reference
CD4 count, mean ±SD, cells/μL		[21]
Treated	259 ± 153	
Untreated	273 ± 231	
Initial HIV RNA level, copies/mL[a]		
> 30,000	71	[21, 22]
10,001–30,000	18	
3,001–10,000	8	
501–3,000	2	
≤500	1	

[a] Data are percentage of patients in the validation cohort with a given HIV RNA level.

Table A2. CD4 count and probability of death for patients awaiting antiretroviral therapy (ART), according to cohort and time before initiating ART.

	Mean CD4 count, mean, cells/μL		Yearly probability of death, %	
Wait before initiating ART	Incident cohort	Prevalent cohort	Incident cohort	Prevalent cohort
1 year	139	38	21.08	44.53
2 years	79	12	33.34	57.90
3 years	38	3	47.74	63.17
4 years	17	1	57.73	64.20
5 years	9	1	61.67	64.66
6 years	6	0	62.69	64.68

Survival and Mortality

Because the baseline characteristics of patients in the prevalent cohort and the incident cohort differed, survival data were derived separately for each cohort. Furthermore, survival data were derived separately for patient populations awaiting ART (who received only co-trimoxazole prophylaxis) and receiving ART.

Patients awaiting ART. We obtained the probabilities of survival for patients awaiting ART by simulating the course of HIV disease with co-trimoxazole prophylaxis only, using the CEPAC International model [14]. Yearly probabilities of survival (conditional on survival to the beginning of the year) were computed by dividing the number of patients alive at the end of the given calendar year by the number alive at the end of the previous year. This was done to reflect a patient's probability of surviving through the year, given that the patient was alive at the

Table A3. Yearly probability of death for patients who received antiretroviral treatment (ART), according to cohort and time before initiating ART.

	Probability of death, %					
Cohort, no. of years of ART	No wait	1-year wait	2-year wait	3-year wait	4-year wait	5-year wait
Incident						
1	6.15	5.09	6.51	8.53	9.97	10.74
2	5.13	4.67	5.07	5.77	6.36	
3	5.10	5.94	6.87	7.83		
4	6.73	7.75	8.48			
5	8.06	8.84				
6	9.05					
Prevalent						
1	5.86	8.67	10.63	11.10	11.20	11.26
2	4.89	5.69	6.62	7.18	7.39	
3	6.59	7.82	8.80	9.48		
4	8.25	9.16	10.05			
5	9.23	10.16				
6	10.24					

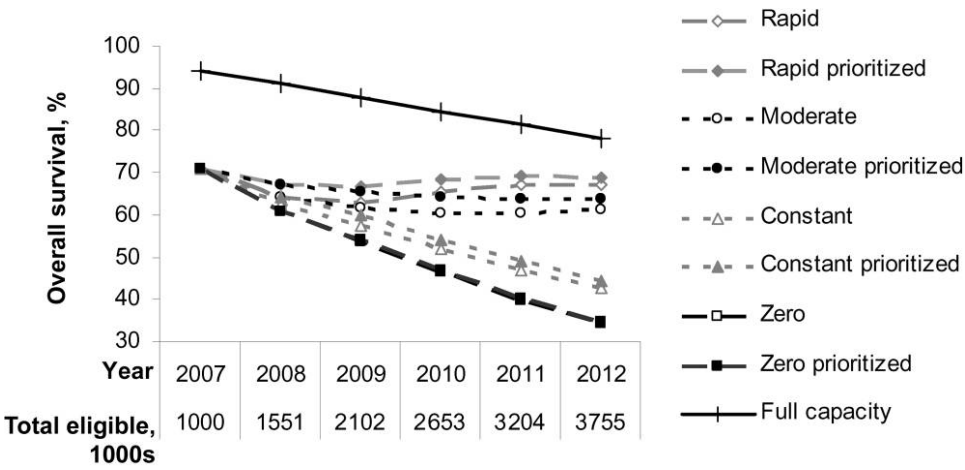

Figure A1. Percentage of total patients eligible for antiretroviral therapy (ART) (both those awaiting and those receiving ART) who were alive by year, for each modeled scenario. The cumulative total of all eligible patients is noted below the graph, by year. The changing denominator and rapid increase in access to ART account for the change in slope of the moderate-growth and rapid-growth curves. Nonprioritized cases are indicated with *open symbols,* and prioritized cases are indicated with *solid symbols.*

beginning of the year. The yearly probabilities of death were defined as the complement of the corresponding yearly probability of survival, as shown in table A2. The first column ("wait before initiating ART") indicates the number of years that patients had been awaiting ART (since the start of the analysis in 2007).

Patients receiving ART. We obtained the probabilities of survival for patients receiving ART by simulating the course of HIV disease using the CEPAC-International model [13]. Because baseline clinical characteristics differed between the prevalent and incident cohorts, mortality rates were derived separately for each cohort. Furthermore, because the baseline clinical characteristics of patients who received ART depended on the duration of time spent awaiting ART, several sets of yearly mortality rates were derived (table A3).

We obtained the baseline characteristics of patients who had awaited ART for varying durations of time (0–5 years) by simulating the course of disease for patients in the prevalent and incident cohorts while receiving co-trimoxazole prophylaxis only, and recording population characteristics at the end of every year (table A2).

We then simulated the course of disease while receiving ART for each distinct patient population described above, as shown in table A3. The first column indicates the patients' cohort and the number of years that patients had been receiving ART since the initiation of the analysis in 2007. Columns 2 through 7 list the probabilities of death for each patient population, depending on the number of calendar years they waited for treatment.

RESULTS

Figure A1 illustrates the population alive, as a percentage of the total population eligible for ART in each year. As an additional 551,000 patients become eligible for ART each year (the incident cohort), the cumulative percentage of patients alive depends on the speed of treatment scale-up. The change in the slope (from downward to upward) in the rapid-growth and moderate-growth curves reflects improved survival rates over time, compared with the increasing total denominator of patients in need (an additional 551,000 patients yearly). By 2012, the rapid-growth and moderate-growth scenarios result in 67% and 61% of the population alive, respectively, compared with the cumulative eligible number (3,755,000). The constant-growth and zero-growth scenarios result in worse cumulative survival rates, with 42% and 34% of the population alive, respectively.

Public-Health and Individual Approaches to Antiretroviral Therapy: Township South Africa and Switzerland Compared

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Abbreviations: 3TC, lamivudine; AZT, zidovudine; CI, confidence interval; d4T, stavudine; EFV, efavirenz; HAART, highly active antiretroviral therapy; IQR, interquartile range; LPV, lopinavir; NFV, nelfinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; TNV, tenofovir

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ABSTRACT

Background

The provision of highly active antiretroviral therapy (HAART) in resource-limited settings follows a public health approach, which is characterised by a limited number of regimens and the standardisation of clinical and laboratory monitoring. In industrialized countries doctors prescribe from the full range of available antiretroviral drugs, supported by resistance testing and frequent laboratory monitoring. We compared virologic response, changes to first-line regimens, and mortality in HIV-infected patients starting HAART in South Africa and Switzerland.

Methods and Findings

We analysed data from the Swiss HIV Cohort Study and two HAART programmes in townships of Cape Town, South Africa. We included treatment-naïve patients aged 16 y or older who had started treatment with at least three drugs since 2001, and excluded intravenous drug users. Data from a total of 2,348 patients from South Africa and 1,016 patients from the Swiss HIV Cohort Study were analysed. Median baseline $CD4^+$ T cell counts were 80 cells/μl in South Africa and 204 cells/μl in Switzerland. In South Africa, patients started with one of four first-line regimens, which was subsequently changed in 514 patients (22%). In Switzerland, 36 first-line regimens were used initially, and these were changed in 539 patients (53%). In most patients HIV-1 RNA was suppressed to 500 copies/ml or less within one year: 96% (95% confidence interval [CI] 95%–97%) in South Africa and 96% (94%–97%) in Switzerland, and 26% (22%–29%) and 27% (24%–31%), respectively, developed viral rebound within two years. Mortality was higher in South Africa than in Switzerland during the first months of HAART: adjusted hazard ratios were 5.90 (95% CI 1.81–19.2) during months 1–3 and 1.77 (0.90–3.50) during months 4–24.

Conclusions

Compared to the highly individualised approach in Switzerland, programmatic HAART in South Africa resulted in similar virologic outcomes, with relatively few changes to initial regimens. Further innovation and resources are required in South Africa to both achieve more timely access to HAART and improve the prognosis of patients who start HAART with advanced disease.

The Editors' Summary of this article follows the references.

Introduction

The introduction of highly active antiretroviral combination therapy (HAART) since 1996 has substantially improved the prognosis of HIV-infected patients in industrialized countries [1]. Only few drugs were available initially, but today over 20 approved antiretroviral drugs from four drug classes are available, including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and fusion inhibitors. In industrialized countries doctors prescribe from the full range of available antiretroviral drugs. Resistance testing and frequent monitoring of CD4 cell counts and viral load are used to individually tailor drug regimens.

In contrast, based on the experience of treating tuberculosis, the World Health Organization (WHO) has developed a public-health approach to providing HAART in resource-limited settings. This approach takes the realities of weak health systems into account, including the level of training of health-care workers, the high patient burden, limited availability of drugs, and the experience of pilot programmes [2,3]. Key characteristics of this public-health approach include the standardisation of first-line and second-line regimens, simplified clinical decision-making, and standardised clinical and laboratory monitoring [3]. The choice of regimens in these programs is determined primarily by cost and ease of administration and can include drugs that are no longer widely used in industrialized countries. Viral load monitoring is not considered essential, and individual drug resistance testing is generally not available. A survey of national guidelines developed by 43 low- and middle-income countries showed that the public-health approach to antiretroviral therapy has been widely adopted in these countries [4]. An estimated 2 million people living with HIV/AIDS were receiving treatment in low- and middle-income countries by December 2006, representing 28% of the estimated 7.1 million people in urgent need of treatment at that time [5].

We compared the public-health and individual approach to HAART by analysing virologic response, changes to first-line regimens, and mortality in patients starting HAART in Switzerland and two townships in Cape Town, South Africa.

Methods

We analysed data from the Khayelitsha and Gugulethu HAART programmes in the Republic of South Africa, which are part of the International Epidemiological Databases to Evaluate AIDS in Southern Africa (IeDEA-SA), and made comparisons with the Swiss HIV Cohort Study (SHCS).

Khayelitsha and Gugulethu Cohorts, Cape Town, South Africa

Khayelitsha and Gugulethu are townships located within the Cape Town metropolitan area with estimated populations of 400,000 and 300,000 people, respectively. Khayelitsha had, in 1999, the first routine government-run programme for the prevention of mother-to-child transmission of HIV in South Africa. Antiretroviral treatment has been available since 2001 at three government clinics providing HIV care, supported by Médecins Sans Frontières. In Gugulethu the Usaphu Luwethu ("Our Family Clinic") antiretroviral treatment programme was initiated by the Desmond Tutu HIV Centre in September 2002. Ten primary-care HIV clinics form the patient referral base.

Enrolment into treatment programmes follows the South African government's Department of Health national guidelines, which are based on the 2002 WHO recommendations [6]. Individuals are eligible for treatment if they are in WHO stage 4 (with the exception of extrapulmonary tuberculosis, which is a stage 4–defining illness but not a criterion for starting therapy in the Western Cape) or have a CD4 count below 200 cells/μl. Data are collected prospectively using structured records completed at each consultation, including information on WHO stage-defining illnesses [7].

In Khayelitsha, viral load assessments are performed routinely before starting HAART, after 3 mo, and then every 6 mo. In Gugulethu viral load is assessed before starting HAART and every 4-mo thereafter. Plasma viral load was measured using a branch DNA hybridization technique (Bayer HIV-1 RNA 3.0 assay, Leverkusen, Germany) or nucleic acid sequence-based amplification (Nuclisens EasyQ assay, bioMérieux, Boxtel, The Netherlands). In South Africa, provincial and national guidelines recommend switching drug regimens after two consecutive viral loads above 5,000 copies/ml, but patients were not necessarily switched when they fulfilled these criteria.

All treatment, clinic consultations, and laboratory work are free of charge. In both sites patients receive counselling and adherence support. Patients on HAART who miss appointments are contacted where possible, and if required, traced through home visits. More details on the Khayelitsha and Gugulethu cohorts are given elsewhere [8,9].

Swiss HIV Cohort Study

Set up in 1988, the Swiss HIV Cohort Study is a national prospective cohort study of HIV-infected patients followed up at outpatient departments of five University hospitals (Basel, Bern, Geneva, Lausanne, and Zurich) and two Cantonal hospitals (Lugano and St. Gallen) in Switzerland. A comparison with official AIDS notifications and deaths indicated that about 70% of all patients living with AIDS in Switzerland participate in the study [10].

Data collection and study procedures are standardised. Detailed information on demographics, mode of HIV acquisition, risk behaviours, clinical events, laboratory results, and treatments is collected at registration and then at intervals of 6 mo. HIV-1 RNA (Roche Amplicor HIV-1 Monitor assay), CD4 counts, and other laboratory parameters are measured at least every 3 mo. Clinical AIDS diagnoses (Centers for Disease Control and Prevention [CDC] stage C) are recorded by the treating physicians on the basis of the 1993 CDC criteria [11]. The decision to change therapy was informed by the International AIDS Society-USA guidelines. All services, including antiretroviral therapy and laboratory testing, are covered by compulsory health insurance. More details on the SHCS are given elsewhere [12,13].

Eligibility Criteria and Definitions

The same eligibility criteria were applied to patients in Switzerland and South Africa. All treatment-naïve patients who started HAART at any point since 2001 (the year when HAART became available in the two South African sites), had at least one day of follow-up, and were aged 16 y or older were included. Patients from Switzerland who acquired HIV

through intravenous drug use were excluded from the analysis because they are a group that is not represented in the South African cohorts. In the South African cohorts HIV transmission information is not routinely recorded, but most patients were infected through heterosexual contacts. HAART was defined as a combination of at least three antiretroviral drugs. The type of regimen was defined as PI-based (two NRTIs and one PI), NNRTI-based (two NRTIs and one NNRTI), and other. Boosted PIs are counted as one drug. The stage of disease was classified as less advanced (CDC stage A/B, WHO stage I/II) or advanced (CDC stage C, WHO stage III/IV).

Ethical Approval and Laboratory Quality Assurance

The local ethics committees of all seven study sites that participate in the SHCS approved the study, and written informed consent was obtained from all participants. Data collection in the townships Khayelitsha and Gugulethu, South Africa, as well as participation of these studies in the Antiretroviral Treatment in Lower Income Countries Collaboration (ART-LINC) collaboration of IeDEA, were approved by the ethics committee of the University of Cape Town, which did not require informed consent. All laboratories involved in South Africa and Switzerland participate in quality assurance programmes.

Endpoints

The following endpoints were considered: time to first treatment change (overall and by reason for change), virologic suppression (defined as HIV-1 RNA $\leq$500 copies/ml), viral rebound (defined as HIV-1 RNA above 500 copies/ml) after having achieved viral suppression, CD4 response, and death from all causes. Viral rebound is usually defined as two consecutive values above a given threshold. In our analysis we used a single value, as the measurement frequency differed between settings. In a sensitivity analysis we used two consecutive values above 500 copies/ml. The threshold of 500 copies/ml was chosen because assays of different sensitivities were used during the study period. Regimen change was defined as any change to the treatment regimen, including interruption and discontinuation, but excluding dosage adjustments. The treating physician indicated the reason for regimen changes, and these reasons were classified as failure (virologic, immunologic, or clinical), toxicity, and other. Specific definitions of reasons for regimen change, including, for example, lactic acidosis, were not standardised across sites. The severity of toxicities was not assessed.

Finally, we determined the proportion of initial regimens that complied with the national South African guidelines or, in the case of Switzerland, the International AIDS Society–USA guidelines current at the time of starting HAART.

Statistical Analysis

We used an "intent-to-continue-treatment" approach and thus ignored changes to treatment, including treatment interruptions and terminations for virological endpoints and death. Time was measured from the start of HAART or from the first viral load measurement of 500 copies/ml or below to the time the outcome occurred, the time of the last follow-up visit, or 2 y after baseline, whichever came first. A patient was considered lost to follow-up if the time between the last visit of the patient and the closing date of the cohort was longer than 1 y. Only patients who potentially had 1 y of follow-up were included in the analysis of loss from follow-up.

We used Kaplan–Meier graphs and Cox proportional hazard models (for mortality and first treatment change) and logistic regression (for viral response and viral rebound) to estimate hazard ratios (HRs) and odds ratios (ORs) of endpoints occurring, in comparing the two South African cohorts with the Swiss cohort. For mortality, a separate model was fitted for the first 3 mo after starting HAART (the period with the highest mortality [14]) and for months 4–24. Since background mortality differs between the two settings, we compared expected mortality rates in Switzerland and South Africa. We obtained estimates of non-HIV-related background mortality by sex and age group from the Global Burden of Disease project [15,16], and used these rates to calculate expected numbers of deaths in the two South African cohorts. Similarly, we used rates available from the Swiss Federal Statistical Office to calculate expected deaths for the Swiss cohort.

For viral response the first viral load measurement within 6–12 mo after baseline was classified into $\leq$500 copies/ml (virologic suppression) or >500 copies/ml. We used logistic regression instead of a time-to-event approach, because the frequency and timing of viral load measurements differed between the two settings. Analyses were adjusted for baseline CD4 count, HIV-1 viral load, stage of disease, sex, and age.

We calculated the rate of changing the first treatment regimen by reason of change and time periods (1–3 mo, 4–6 mo, 7–12 mo, and 13–24 mo) by dividing the number of patients developing the event by the number of person-years at risk. We used Poisson regression to calculate confidence intervals (CIs) for rates. Cause-specific cumulative incidences were calculated applying a competing risk approach [17,18]. We used competing risk Cox regression as described by Lunn and McNeil [19] to jointly analyze treatment change due to failure, intolerance, and other reasons. This analysis was adjusted for the same variables as above.

All analyses were performed using Stata version 9.2. Results are presented as Kaplan–Meier probabilities, rates per 100 person-years, and HRs or ORs, with 95% CIs.

Results

Patient characteristics

A total of 3,364 patients—2,348 from Khayelitsha and Gugulethu and 1,016 from the Swiss HIV Cohort Study—were observed for 2,362 and 1,564 person-years, respectively. The median observation time (taking censoring after 2 y into account) was 0.7 y (interquartile range [IQR] 0.3–1.3 y) for Gugulethu, 1.0 years (0.5–1.5 y) for Khayelitsha and 2.0 y (1.1–2.0 y) for the Swiss cohort. Table 1 shows the characteristics of patients at the time of treatment initiation. The patients in the South African cohorts were younger, more likely to be female, and in more advanced stages of the infection: the median baseline CD4 count was 80 cells/μl compared to 204 cells/μl in the Swiss cohort, and 2,126 (90.6%) and 188 (18.5%), respectively, were in CDC stage C or WHO stage III/IV. In South Africa the number of patients starting HAART almost doubled every year from 79 in 2001 to 509 in 2003, whereas in Switzerland the number of patients starting HAART remained fairly constant since 2001.

Table 1. Baseline Characteristics of Patients Starting HAART in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study

Category	Subcategory	South African Cohorts	Swiss HIV Cohort
Patients, *n*	—	2,348	1,016
	Females, *n* (%)	1,663 (71%)	333 (33%)
Age, y, median (IQR)	—	32.7 (28.5–38.2)	38.3 (31.7–46.1)
CD4 cell count, cells/μl, median (IQR)	—	80 (30–138)	204 (122–291)
CD4 cell count, number missing (%)	—	123 (5.2%)	50 (4.9%)
HIV-1 viral load, $\log_{10}$ copies/ml, median (IQR)	—	5.1 (4.6–5.5)	5.0 (4.5–5.5)
HIV-1 viral load, number missing (%)	—	288 (12.3%)	53 (5.2%)
Clinical stage: CDC stage C, WHO stage III/IV, *n* (%)	—	2,126 (90.6%)	188 (18.5%)
Duration of follow-up (years)[a], median (IQR)	—	0.9 (0.5–1.5)	2.0 (1.1–2.0)
Number of patients starting HAART in year, *n* (%)	2001	79 (3.4%)	179 (17.6%)
	2002	246 (10.5%)	204 (20.1%)
	2003	509 (21.7%)	185 (18.2%)
	2004–2006[b]	1,514 (64.5%)	448 (44.1%)
Initial HAART regimen, *n* (%)	2 NRTIs + 1 NNRTI	2,339 (99.7%)	470 (46.3%)
	2 NRTIs + 1 PI	9 (0.4%)	449 (44.2%)
	Other	0	97 (9.6%)
Four most commonly used initial HAART regimens, *n* (%)	3TC D4T EFV	918 (39.1%)	—
	3TC D4T NVP	780 (33.2%)	—
	3TC AZT EFV	425 (18.1%)	—
	3TC AZT NVP	216 (9.2%)	—
	3TC AZT EFV	—	304 (29.9%)
	3TC AZT LPV/r	—	202 (19.9%)
	3TC AZT NFV	—	99 (9.7%)
	3TC TNV EFV	—	65 (6.4%)
Initial HAART regimens used to treat 95% of patients, *n*	—	4	36
Patients using initial HAART regimens recommended by guidelines, %	—	95.1	99.6

[a]Censored after 2 y.
[b]Recruitment not completed for the years 2005/06.
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In Khayelitsha and Gugulethu over 95% of patients were treated with one of four NNRTI-based first-line regimens whereas in Switzerland 36 different regimens were used (Table 1). The most commonly used regimens in South Africa were stavudine/lamivudine (d4T/3TC) in combination with efavirenz (EFV) (39.1%) or nevirapine (NVP) (33.2%). In Switzerland the most frequent regimens were zidovudine/lamivudine (AZT/3TC) in combination with either EFV ($n = 304$, 29.9%) or boosted lopinavir (LPV) ($n = 202$, 19.9%) or nelfinavir (NFV) ($n = 99$, 9.7%). Stavudine (d4T) was used in 35 patients (3.4%) only. In South Africa 2,339 (99.6%) of patients started with a regimen that was in accordance with guidelines, compared to 966 (95.1%) of patients in Switzerland. In Switzerland 45 patients (4.5%) received regimens that may have been chosen because of primary resistance or as part of a study, and five patients (0.5%) received regimens that clearly violated guidelines.

Changes to First-Line Regimens

Changes to the initial regimen during the first 2 y of HAART were considerably more frequent in Switzerland than in South Africa: 539 patients (53.1%) compared to 514 patients (21.9%) experienced at least one change during the first 2 y of HAART. Substitutions of one drug were the most frequent change both in Switzerland (274, 50.8%) and South Africa (328, 63.8%). Figure 1 shows rates of any type of change during months 1–3, 4–6, 7–12, and 13–24 along with the estimated cumulative probability of change, by reason for changing regimens: toxicity, failure, or other reasons. The cumulative probability of change at 2 y due to toxicity was 23.8% (95% CI 21.0%–26.7%) in Switzerland compared to 11.7% (95% CI 10.0%–13.5%) in Khayelitsha and Gugulethu. In contrast, the probability of changes due to failure was similar in Switzerland and South Africa: 5.1% (95% CI 3.7%–6.8%) and 3.9% (2.5%–5.6%), respectively. In patients who were switched for failure, the median $\log_{10}$ HIV viral load values at the time of treatment switch due to failure was 4.4 $\log_{10}$ copies/ml in South Africa ($n = 31$) and 3.4 $\log_{10}$ copies/ml in Switzerland ($n = 39$) ($p < 0.001$ for difference). In South Africa all patients who switched for failure had detectable viral loads, whereas in Switzerland ten patients (29%) had viral loads $\leq$500 copies/ml. An estimated 30.9% (95% CI 27.7%–34.1%) of patients had changed regimens for other reasons in Switzerland compared to 14.1% (12.1%–16.3%) in South Africa.

In both settings toxicity was the dominant reason for treatment change in the first 3 mo, but rates were considerably higher in Switzerland than in South Africa: 53 per 100 person-years (95% CI 44–63) compared to 21 per 100 person-years (17–25). Other reasons dominated from month 4 onwards, again with higher rates of change in Switzerland. Treatment failure as reason for drug changes was rare in both settings. Table 2 compares reasons for regimen change in more detail. For toxicity the most notable difference relates to elevated lactate levels and lactic acidosis: in Khayelitsha and Gugulethu 32 (13.4%) of all regimen changes due to toxicities were due to lactic acidosis (associated with d4T in 31 patients). In the Swiss study no patient changed the initial regimen due to elevated lactate levels. Treatment changes due to abdominal and gastrointestinal toxicity, including liver toxicity, were more common in Switzerland than in South

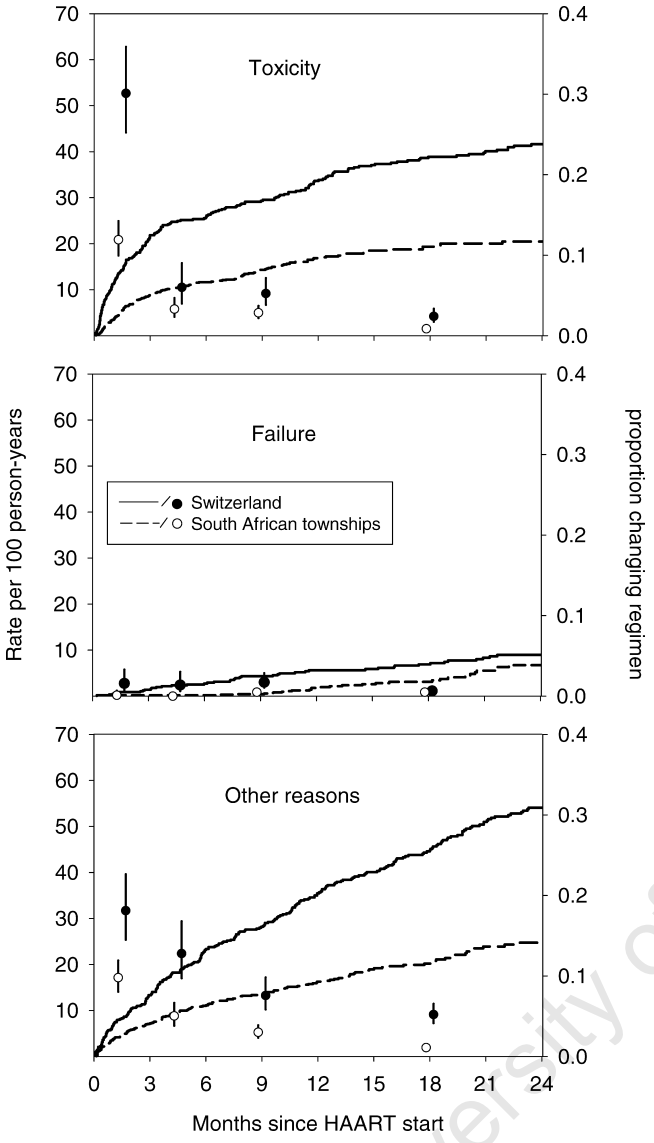

Figure 1. Rates and Kaplan-Meier Plots of First Treatment Change Due to Toxicity, Failure, and Other Reasons in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study

Dots indicate rates during months 1–3, 4–6, 7–12, and 13–24 with 95% CIs; lines indicate the estimated proportion of patients changing their first-line regimen. "Other reasons" (bottom graph) include mainly treatment changes due to tuberculosis and pregnancy in South Africa and changes due to patients' wishes or physicians' decisions in Switzerland.

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Africa. Changes due to other reasons were also more frequent in Switzerland. The predominant other reasons for changing first-line regimens in South Africa were pregnancy and tuberculosis. In Switzerland changes due to patient requests were common.

Virologic Response, Rebound, CD4 Response, and Mortality

The frequency of HIV-1 RNA measurements differed in the two settings (Figure 2). The median time to the first HIV-1 RNA determination was 3.1 mo (interquartile range [IQR] 2.8–3.7 mo) in Khayelitsha and Gugulethu compared to 1.0 mo (0.7–1.5 mo) in the Swiss study. During follow-up HIV-1 RNA was measured at regular time intervals in South Africa (median 3.9 mo, IQR 3.2–6.0 mo) whereas in Switzerland the time intervals were not well defined (2.9 mo, 1.9–3.4 mo). A similar picture was evident for CD4 counts (unpublished data). Kaplan-Meier plots show that in both settings most patients suppressed HIV-1 RNA to 500 copies/ml or less (Figure 3, top graph) within one year: 96.0% (95% CI 95.1%–96.9%) of patients in the townships and 95.5% (94.0%–96.7%) in the Swiss cohort. The proportion of patients with viral load values $\leq$500 copies/ml at different time points was around 90% up to 2 y in both Switzerland and South Africa.

Among the 2,644 patients who suppressed viral replication to $\leq$500 copies/ml (1,716 in South Africa and 928 in Switzerland), the probability of a viral rebound at 2 y after suppression was 25.5% (95% CI 22.1%–29.3%) in South Africa and 27.1% (23.9%–30.7%) in Switzerland (Figure 3, middle graph). When analyses were repeated with two consecutive measurements above 500 copies/ml, the rate of viral rebound was slightly higher in Switzerland than in South Africa, which is expected considering the higher measurement frequency in Switzerland. During the 2 y the median CD4 count increased from 80 cells/μl at baseline (IQR 30–138) to 372 cells/μl (260–497) in South Africa and from 204 cells/μl (122–291) to 449 (310–607) in Switzerland. Patients starting HAART with lower CD4 cell counts tended to have lower values throughout the study period, both in South Africa and Switzerland.

Mortality was substantially higher in South Africa than in Switzerland during the first months of HAART (Figure 3, bottom graph). Cumulative mortality at 6 mo was 8.6% (95% CI 7.5%–9.8%) and 0.9% (0.5%–1.8%), respectively. The proportion of patients lost to follow-up was similar: by 1 y, 3.5% (95% CI 2.5%–4.7%) of patients in Khayelitsha and Gugulethu and 3.2% (2.2%–4.7%) of patients in the Swiss cohort were lost to follow-up.

Univariable and Multivariable Analyses

The results from univariable and multivariable logistic and Cox models comparing the South African cohorts with the Swiss cohort are presented in Table 3. For the three endpoints change from first-line regimen, virologic response and viral rebound, adjusting the models for sex and age, CD4 cell count, HIV-1 RNA, and stage of disease at baseline had only modest effects on HRs and ORs. The adjusted HRs comparing South Africa with Switzerland for treatment change due to failure, intolerance, and other reasons were 0.25 (95% CI 0.12–0.50), 0.44 (0.32–0.60), and 0.30 (0.22–0.40) respectively, but there was little evidence for a difference in virologic response and viral rebound (Table 3). In contrast, HRs for the mortality endpoints were attenuated considerably in multivariable analysis: the adjusted HRs were 5.90 (95% CI 1.81–19.21) during months 1–3 and 1.77 (0.90–3.50) during months 4–24. The expected non-HIV-related mortality rate was 28.1 per 10,000 person-years in the South African cohorts compared to 13.3 in the Swiss cohort, for a rate ratio of 2.11 (95% CI 1.10–4.06).

Discussion

This comparative study of patients starting HAART in South Africa and Switzerland found that the initial virologic response was similar, despite profound differences in patient

Table 2. Reasons for Change of the First HAART Regimen in the First Two Years of Treatment in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study

Reason	Details	South African Cohorts ($n = 514$)	Swiss Cohort ($n = 539$)
		Patients, *n* (%)	Patients, *n* (%)
Toxicity	Any	238 (46.3%)	220 (40.8%)
	Peripheral neuropathy	46 (8.9%)	53 (9.8%)
	Gastrointestinal, including liver	37 (7.2%)	64 (11.9%)
	Haematological	36 (7.0%)	20 (3.7%)
	Lactic acidosis	32 (6.2%)	0 (0%)
	Hypersensitivity[a]	30 (5.8%)	34 (6.3%)
	Lipodystrophy	6 (1.2%)	12 (2.2%)
	Dislipidaemia	4 (0.8%)	5 (0.9%)
	Nephrological	0 (0%)	7 (1.3%)
	Not specified	47 (9.1%)	25 (4.6%)
Failure	Virologic, immunologic, or clinical	31 (6.0%)	39 (7.2%)
Other	—[b]	244 (47.5%)	257 (47.7%)
Unknown	—	1 (0.2%)	23 (4.3%)

[a]Any type of hypersensitivity in Switzerland, rash in South Africa.
[b]In South Africa "other" reasons mainly included contraindications related to tuberculosis or pregnancy (167 patients); in Switzerland physician decisions (including contraindications, changes in guidelines, etc.; 107 patients) and patient requests (85 patients).
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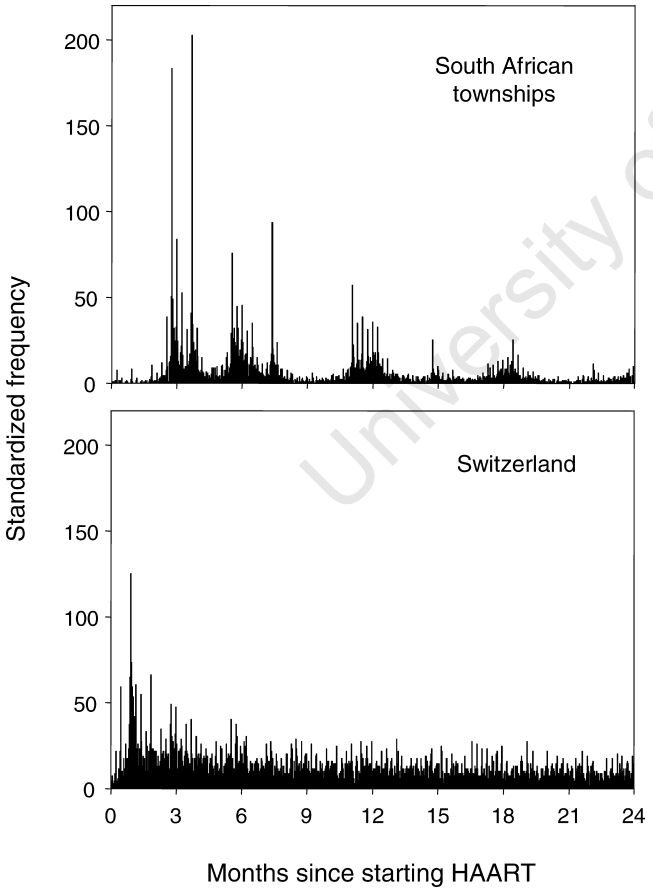

Figure 2. Frequency of Viral Load Measurements in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study
The frequency was standardized to the total number of measurements in each setting.
doi:10.1371/journal.pmed.0050148.g002

characteristics and the approach to antiretroviral therapy, and different viral strains causing the epidemics in the two countries. Compared to South Africa, about twice as many changes to the treatment regimen were recorded in Switzerland during the first two years. Mortality was higher in South Africa than in Switzerland, particularly during the first three months of HAART.

Public Health and Individualised Approaches to HAART

In South Africa, where the prevalence of HIV-1 subtype C infection in the general population is estimated at 19%, the Department of Health published detailed treatment guidelines for adults and children in 2004 [6], with the objective of providing access to all patients in need. All patients start a regimen consisting of a recommended NRTI backbone and either EFV or NVP. In December 2006, an estimated 1,000,000 people in South Africa needed HAART, and 325,000 were receiving it [5]. In contrast, in Switzerland the prevalence of HIV-1 is below 1% and mainly of subtype B, HAART is covered by the compulsory basic health insurance package, and access is therefore universal. Care is highly individualised and delivered by specialists in HIV medicine. The choice of the initial regimen is influenced by several factors, including convenience, viral resistance to treatment, potential side effects, and physician and patient preferences. In both countries the provision of HAART has been found to be cost-effective from a health services and societal perspective [20–22].

Antiretroviral Anarchy?

There has been concern that unregulated use of antiretroviral drugs, interruptions in drug supplies, and the lack of monitoring of treatment response in sub-Saharan Africa might lead to "antiretroviral anarchy" and the emergence of viral resistance [23]. For example, in Abidjan, Côte d'Ivoire, 39 (57%) of 68 patients who had relied on friends or relatives in Europe or the United States for antiretroviral drugs before a HAART programme was established had mutations in their

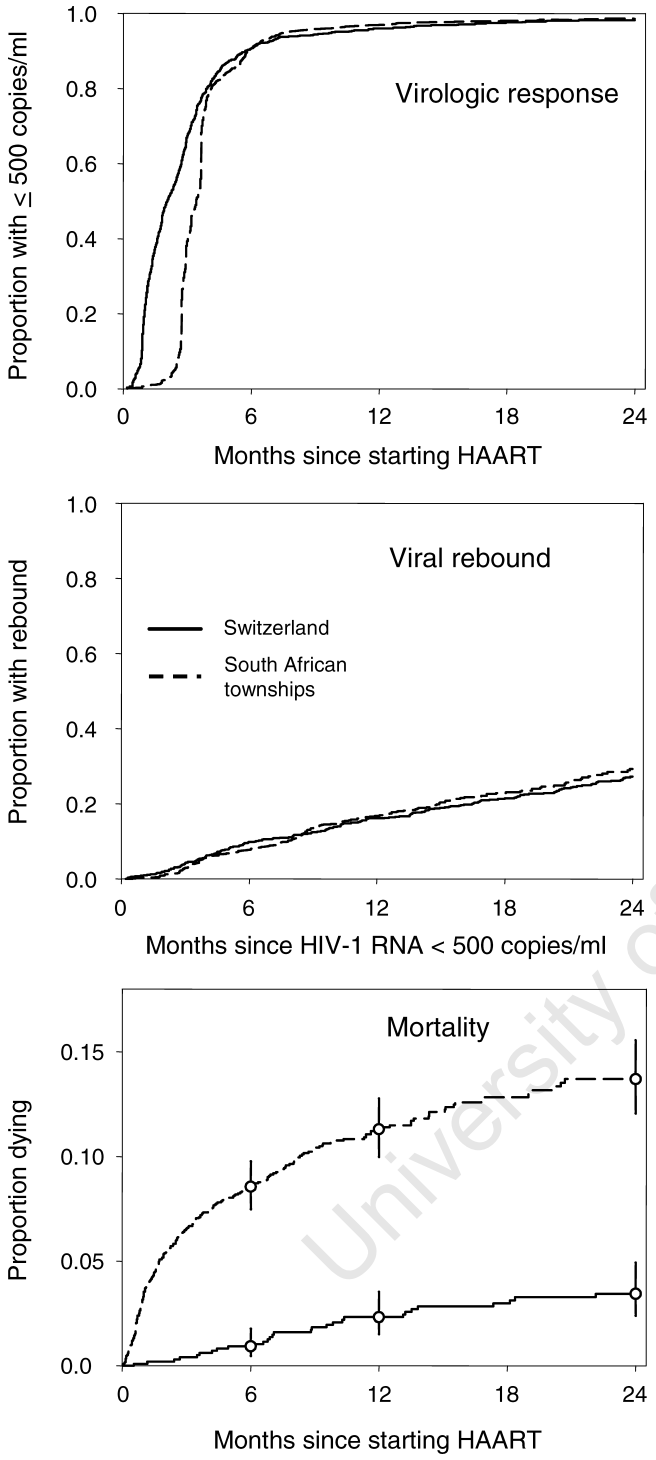

Figure 3. Kaplan Meier Plots of Virologic Response, Viral Rebound, and Mortality in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study

Viral rebound is defined as having a HIV-1 RNA >500 copies/ml after a viral load ≤500 copies/ml.

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virus associated with resistance to at least one drug [24]. Similar data were reported from Libreville, Gabon [25]. The development of resistance is closely linked to incomplete adherence to therapy, and several studies have shown that good adherence can be achieved in resource-limited settings [26–28]. The overall virologic response observed in this study suggests that adherence was good in the two South African townships (the delay in reaching viral load values below 500 copies/ml in South Africa is probably explained by the less-frequent viral load determinations in the township cohorts compared to Switzerland). Therefore, our results indicate that antiretroviral anarchy has been prevented in township ART programmes in South Africa.

In industrialized settings, a substantial proportion of new infections now involve strains resistant to one or more drugs [29]. Viral resistance is rare in South Africa at present [30], but is bound to increase in the future. WHO monitors drug resistance at sentinel sites in South Africa and elsewhere [3]. The use of single-dose NVP to prevent mother-to-child transmission of HIV may increase resistance levels, but the implications for treatment are a matter of debate and the subject of ongoing studies [31].

Considering the large number of first-line regimens used in Switzerland, and the high rate of changes to these regimens, one might argue that antiretroviral anarchy may in fact be more prevalent in Switzerland than in South Africa. However, we found that 95% of patients used regimens that were in accordance with the International AIDS Society–USA guidelines in place during this period. Few regimens violated the current guidelines. Nevertheless, a more standardised approach to the choice of the first-line regimen and monitoring of viral load could probably reduce costs in Switzerland without compromising the effectiveness of HAART.

Treatment Changes

Treatment changes that were reported to be due to toxicity in the first 3 mo of treatment were more frequent in Switzerland than in South Africa, despite the fact that in Switzerland more drugs, and more drugs with a more favourable adverse effects profile, are available. The type of toxicities leading to treatment changes were fairly similar in the two settings, with the exception of symptomatic hyperlactataemia or lactic acidosis, which was recorded in 32 patients in South Africa but not observed in Switzerland. This difference is not surprising in light of the widespread use of stavudine in South Africa but not in Switzerland. In South Africa few patients switched because of lipodystrophy, despite the widespread use of stavudine, possibly because follow-up was relatively short. Indeed, a previous analysis of the Khayelitsha and Gugulethu cohorts showed that drug substitutions due to lipodystrophy occurred mainly after the first year of treatment [32]. We stress that our analysis was restricted to treatment changes attributed to toxicities: we did not assess the overall incidence of toxicities nor the severity of adverse events.

Early Mortality

Patients in South Africa started therapy with much more pronounced immunodeficiency than did those in Switzerland, reflecting the large number of patients in great need of treatment during the scale-up of HAART in South Africa. In line with a previous collaborative analysis [14], the higher mortality in the South African townships was probably only partly explained by lower CD4 cell counts and more advanced clinical stage at treatment initiation. It seems likely that specific comorbidities, including invasive bacterial and fungal infections, are important in this context. For example, an

Table 3. Unadjusted and Adjusted HRs and ORs for Study Endpoints, Comparing Khayelitsha and Gugulethu, South Africa, with the Swiss HIV Cohort Study

Study Endpoints	Number of Patients, South Africa/Switzerland	Number of Events, South Africa/Switzerland	Unadjusted HRs or ORs (95% CI)	*p*-Value	Adjusted[a] HRs or ORs (95% CI)	*p*-Value
First treatment changed	1,972/959	514/539	—	—	—	—
First treatment change due to failure	—	31/39	0.33 (0.20–0.53)	<0.001	0.25 (0.12–0.50)	<0.001
First treatment change due to intolerance	—	238/220	0.43 (0.36–0.52)	<0.001	0.44 (0.32–0.60)	<0.001
First treatment change due to other reasons	—	244/257	0.37 (0.31–0.45)	<0.001	0.30 (0.22–0.40)	<0.001
Viral suppression[b]	1,175/810	1,074/735	1.09 (0.79–1.48)	0.61	1.14 (0.67–1.93)	0.63
Viral rebound	862/783	74/52	1.32 (0.91–1.91)	0.14	1.26 (0.68–2.31)	0.46
Mortality (months 1–3)	1,972/959	118/4	14.60 (5.39–39.54)	<0.001	5.90 (1.81–19.21)	0.003
Mortality (months 4–24)	1,823/912	85/23	2.61 (1.64–4.15)	<0.001	1.77 (0.90–3.50)	0.10

HRs are given for mortality and treatment change, ORs for initial viral response and viral rebound. The difference in the number of patients included in these analyses compared to the total number of patients shown in Table 1 is explained by missing values in key variables.

[a]Analyses were adjusted for sex, age, baseline CD4 cell count, baseline HIV viral load and stage of disease.

[b]HIV-1 RNA ≤500 copies/ml.

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earlier analysis of the Gugulethu data showed that six (27%) of the 22 deaths that occurred during the first 3 mo were due to cryptococcal disease, with a clinical course suggestive of immune reconstitution disease [33].

Unfortunately, causes of death are not recorded systematically in the South African cohorts. A study from rural Uganda showed that a positive serum cryptococcal antigen was associated with substantially increased early mortality (adjusted relative risk 6.6; 95% CI 1.9–12.6) [34]. The corresponding relative risk for active tuberculosis was 4.4 (95% CI 1.2–15.4) [34]. Clinical trials in South Africa and elsewhere will help identify strategies to reduce mortality, including, for example, trials of HAART initiation in HIV–TB coinfected patients [35] and isoniazid preventive therapy in patients receiving HAART [36]. Limited access to diagnostic tests, procedures, and drugs to diagnose and treat opportunistic illnesses, including access to intensive care, may also have contributed to the higher mortality in the South African townships.

After the first few months of HAART, mortality was low in both the South African and Swiss cohorts. The higher mortality in South Africa during this period probably reflects a higher (non-HIV-related) background mortality in South Africa. This interpretation is supported by our comparison of mortality in Switzerland with non-HIV-related mortality in South Africa. South African national HAART programme still bases its treatment guidelines on the 2002 WHO guidelines, which recommend HAART only for patients with WHO stage IV disease or a CD4 cell count of less than 200 cells/μl [37]. These recommendations were revised in 2003 and now state that in patients with WHO stage III disease, treatment should be considered when the CD4 count is below 350 cells/μl and initiated before the CD4 count drops to below 200 cells/μl [38]. Recent analyses from Cape Town showed high mortality before HAART is started or before a formal AIDS diagnosis is made [39–41]. Taken together, there is strong evidence that public health strategies to increase access in South Africa should be further promoted. A recent analysis of the Swiss cohort showed that in Switzerland, late presentation is the reason for late initiation of HAART: once the diagnosis is made, uptake of HAART is fast [42].

Strengths and Limitations of the Study

All patients in this study were treatment-naïve at the start of HAART, and results are therefore not affected by previous antiretroviral therapy.

The two South African township programmes were part of the first public HAART programmes in Southern Africa and are typical of many sites involved in the scale-up of HAART in this region. Scale-up is reflected in the rapid increase in the number of patients starting therapy during the study period. Our study did not, however, include the private sector in South Africa, and no comparison has been made between the overall quality of medical care to support HAART and HIV disease management that is likely to have an important impact on mortality. The SHCS was one of the first HIV cohort studies worldwide [12], and it includes all major HIV outpatient clinics as well as a number of large private practices. It is estimated that about 40% of all patients with HIV and about 70% of patients with AIDS are included [10].

A limitation of our study is that although the reasons for changes in therapy were assessed prospectively, this was not done using the same, standardised instrument and definitions in South Africa and Switzerland. The attribution of the causes for treatment change is further complicated by the fact that causes are not independent: a patient might want to change therapy due to side effects, or side effects can cause problems with adherence, which then leads to treatment failure.

The rate of loss to follow-up was low: in both settings patients who missed appointments were contacted and, if required, traced. However, follow-up information for the South African sites is limited due to the continuous scale-up: i.e., the majority of patients were registered only recently and were thus followed only over a short period of time. These patients will, by definition, not be lost to follow-up. Continued follow-up of patients in South Africa is needed to allow comparisons of treatment responses over longer periods of time.

Conclusions

A public-health approach to HAART provision using a limited repertoire of drugs and relatively few viral load measurements resulted in virologic treatment outcomes in townships in South Africa that were similar to outcomes in Switzerland. Our study also shows that many patients would benefit from earlier initiation of therapy, particularly in South Africa.

Supporting Information

Alternative Language Abstract S1. The Abstract Translated into French by Claire Graber

Found at doi:10.1371/journal.pmed.0050148.sd001 (26 KB DOC).

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Author contributions. OK, ME, and AB designed the study. CO, RW, HF, GvC, and AB collected data for the study and enrolled patients. OK wrote the first draft of the paper, which was subsequently revised by CO and ME. All authors contributed to the final version of the paper. CO collected and entered all the data from the South African Gugulethu site. ME, MWGB, BL, and AB advised on the statistical analyses, which were done by OK. GvC represents the Khayelitsha cohort collaboration team, which includes staff from Médecins Sans Frontières, the Infectious Disease Epidemiology Unit, and the Provincial Government of the Western Cape.

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Editors' Summary

Background. Acquired immunodeficiency syndrome (AIDS) has killed more than 25 million people since the first reported case in 1981, and more than 30 million people are now infected with the human immunodeficiency virus (HIV), which causes AIDS. HIV destroys immune system cells (including CD4 cells, a type of lymphocyte), leaving infected individuals susceptible to other infections. Early in the AIDS epidemic, most HIV-infected people died within 10 years of becoming infected. Then, in 1996, highly active antiretroviral therapy (HAART)—a combination of several antiretroviral drugs—was developed. Now, in resource-rich countries, clinicians provide individually tailored care for HIV-infected people by prescribing combinations of antiretroviral drugs chosen from more than 20 approved medicines. The approach to treatment of HIV in developed countries typically also includes frequent monitoring of the amount of virus in patients' blood (viral load), viral resistance testing (to see whether any viruses are resistant to specific antiretroviral drugs), and regular CD4 cell counts (an indication of immune-system health). Since the implementation of these interventions, the health and life expectancy of people with HIV has improved dramatically in these countries.

Why Was This Study Done? The history of HIV care in resource-poor countries has been very different. Initially, these countries could not afford to provide HAART for their populations. In 2003, however, governments, international agencies, and funding bodies began to implement plans to increase HAART coverage in developing countries. By December 2006, more than a quarter of the HIV-infected people in low- and middle-income countries who urgently needed treatment were receiving HAART. However, instead of individualized treatment, HAART programs in developing countries follow a public-health approach developed by the World Health Organization. That is, drug regimens, clinical decision-making, and clinical and laboratory monitoring are all standardized. This public-health approach takes into account the realities of under-resourced health systems, but is it as effective as the individualized approach? The researchers addressed this question by comparing virologic responses (the effect of treatment on the viral load), changes to first-line (initial) therapy, and deaths in patients receiving HAART in South Africa (public-health approach) and in Switzerland (individualized approach).

What Did the Researchers Do and Find? The researchers analyzed data collected since 2001 from more than 2,000 patients enrolled in HAART programs in two townships (Gugulethu and Khayelitsha) in Cape Town, South Africa, and from more than 1,000 patients enrolled in the Swiss HIV Cohort Study, a nationwide study of HIV-infected people. The patients in South Africa, who had a lower starting CD4 cell count and were more likely to have advanced AIDS than the patients in Switzerland, started their treatment for HIV infection with one of four first-line therapies, and about a quarter changed to a second-line therapy during the study. By contrast, 36 first-line regimens were used in Switzerland and half the patients changed to a different regimen. Despite these differences, the viral load was greatly reduced within a year in virtually all the patients and viral rebound (an increased viral load after a low measurement) developed within 2 years in a quarter of the patients in both countries. However, more patients died in South Africa than in Switzerland, particularly during the first 3 months of therapy.

What Do These Findings Mean? These findings suggest that the public-health approach to HAART practiced in South Africa is as effective in terms of virologic outcomes as the individualized approach practiced in Switzerland. This is reassuring because it suggests that "antiretroviral anarchy" (the unregulated use of antiretroviral drugs, interruptions in drug supplies, and the lack of treatment monitoring), which is likely to lead to the emergence of viral resistance, is not happening in South Africa as some experts feared it might. Thus, these findings support the continued rollout of the public-health approach to HAART in resource-poor countries. Conversely, they also suggest that a more standardized approach to HAART could be taken in Switzerland (and in other industrialized countries) without compromising its effectiveness. Finally, the higher mortality in South Africa than in Switzerland, which partly reflects the many patients in South Africa in desperate need of HAART and their more advanced disease at the start of therapy, suggests that HIV-infected patients in South Africa and in other resource-limited countries would benefit from earlier initiation of therapy.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0050148.

- The World Health Organization provides information about universal access to HIV treatment (in several languages) and on its recommendations for a public-health approach to antiretroviral therapy for HIV infection
- More details on the Swiss HIV Cohort Study and on the studies in Gugulethu and Khayelitsha are available
- Information is available from the US National Institute of Allergy and Infectious Diseases on HIV infection and AIDS
- HIV InSite has comprehensive information on all aspects of HIV/AIDS, including detailed information about antiretroviral therapy and links to treatment guidelines for various countries
- Information is available from Avert, an international AIDS charity, on HIV and AIDS around the world and on providing AIDS drug treatment for millions

Correction: Public-Health and Individual Approaches to Antiretroviral Therapy: Township South Africa and Switzerland Compared

Olivia Keiser, Catherine Orrell, Matthias Egger, Robin Wood, Martin W. G. Brinkhof, Hansjakob Furrer, Gilles van Cutsem, Bruno Ledergerber, Andrew Boulle, for the Swiss HIV Cohort Study (SHCS) and the International Epidemiologic Databases to Evaluate AIDS in Southern Africa (IeDEA-SA)

Correction for:
Keiser O, Orrell C, Egger M, Wood R, Brinkhof MWG, et al. (2008) Public-health and individual approaches to antiretroviral therapy: Township South Africa and Switzerland compared. PLoS Med 5(7): e148. doi:10.1371/journal.pmed.0050148

The Table 1 originally published with this manuscript stated that 95.1% of the patients from South Africa used regimens recommended by guidelines, compared to 99.6% in the Swiss cohort. This is incorrect: the figures have been reversed. As the article text makes clear, 95.1% of the Swiss patients used regimens recommended by guidelines, compared to 99.6% in the South African cohorts.

A corrected version of Table 1 is published here.

Table 1. Baseline Characteristics of Patients Starting HAART in Khayelitsha and Gugulethu, South Africa and the Swiss HIV Cohort Study

Category	Subcategory	South African Cohorts	Swiss HIV Cohort
Patients, *n*	—	2,348	1,016
	Females, *n* (%)	1,663 (71%)	333 (33%)
Age, y, median (IQR)	—	32.7 (28.5–38.2)	38.3 (31.7–46.1)
CD4 cell count, cells/μl, median (IQR)	—	80 (30–138)	204 (122–291)
CD4 cell count, number missing (%)	—	123 (5.2%)	50 (4.9%)
HIV-1 viral load, $\log_{10}$ copies/ml, median (IQR)	—	5.1 (4.6–5.5)	5.0 (4.5–5.5)
HIV-1 viral load, number missing (%)	—	288 (12.3%)	53 (5.2%)
Clinical stage: CDC stage C, WHO stage III/IV, *n* (%)	—	2,126 (90.6%)	188 (18.5%)
Duration of follow-up (years)[a], median (IQR)	—	0.9 (0.5–1.5)	2.0 (1.1–2.0)
Number of patients starting HAART in year, *n* (%)	2001	79 (3.4%)	179 (17.6%)
	2002	246 (10.5%)	204 (20.1%)
	2003	509 (21.7%)	185 (18.2%)
	2004–2006[b]	1,514 (64.5%)	448 (44.1%)
Initial HAART regimen, *n* (%)	2 NRTIs + 1 NNRTI	2,339 (99.7%)	470 (46.3%)
	2 NRTIs + 1 PI	9 (0.4%)	449 (44.2%)
	Other	0	97 (9.6%)
Four most commonly used initial HAART regimens, *n* (%)	3TC D4T EFV	918 (39.1%)	—
	3TC D4T NVP	780 (33.2%)	—
	3TC AZT EFV	425 (18.1%)	—
	3TC AZT NVP	216 (9.2%)	—
	3TC AZT EFV	—	304 (29.9%)
	3TC AZT LPV/r	—	202 (19.9%)
	3TC AZT NFV	—	99 (9.7%)
	3TC TNV EFV	—	65 (6.4%)
Initial HAART regimens used to treat 95% of patients, *n*	—	4	36
Patients using initial HAART regimens recommended by guidelines, %	—	99.6	95.1

[a]Censored after 2 y.
[b]Recruitment not completed for the years 2005/06.
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Expanding Antiretroviral Options in Resource-Limited Settings—A Cost-Effectiveness Analysis

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Background: Current World Health Organization (WHO) guidelines for treatment of HIV in resource-limited settings call for 2 antiretroviral regimens. The effectiveness and cost-effectiveness of increasing the number of antiretroviral regimens is unknown.

Methods: Using a simulation model, we compared the survival and costs of current WHO regimens with two 3-regimen strategies: an initial regimen of 3 nucleoside reverse transcriptase inhibitors followed by the WHO regimens and the WHO regimens followed by a regimen with a second-generation boosted protease inhibitor (2bPI). We evaluated monitoring with CD4 counts only and with both CD4 counts and viral load. We used cost and effectiveness data from Cape Town and tested all assumptions in sensitivity analyses.

Results: Over the lifetime of the cohort, 25.6% of individuals failed both WHO regimens by virologic criteria. However, when patients were monitored using CD4 counts alone, only 6.5% were prescribed additional highly active antiretroviral therapy due to missed and delayed detection of failure. The life expectancy gain for individuals who took a 2bPI was 6.7–8.9 months, depending on the monitoring strategy. When CD4 alone was available, adding a regimen with a 2bPI was associated with an incremental cost-effectiveness ratio of $2581 per year of life gained, and when viral load was available, the ratio was $6519 per year of life gained. Strategies with triple–nucleoside reverse transcriptase inhibitor regimens in initial therapy were dominated. Results were sensitive to the price of 2bPIs.

Conclusions: About 1 in 4 individuals who start highly active antiretroviral therapy in sub-Saharan Africa will fail currently recommended regimens. At current prices, adding a regimen with a 2bPI is cost effective for South Africa and other middle-income countries by WHO standards.

(*J Acquir Immune Defic Syndr* 2009;00:000–000)

INTRODUCTION

Africa is home to more than 20 million HIV-infected individuals, two thirds of all new infections, and three quarters of all HIV-related deaths.[1] By the end of 2007, more than 2 million people have initiated highly active antiretroviral therapy (HAART) in sub-Saharan Africa and access to treatment continues to expand.[2] However, HIV treatment in publicly funded programs in sub-Saharan Africa is restricted to 2 regimens.[3,4] The World Health Organization (WHO) provides no guidelines beyond second-line therapy, and individuals who fail or cannot tolerate first-line and second-line regimens do not have access to further antiretroviral treatment options.[4] In contrast, 6 different classes of antiretrovirals are available in developed countries yielding multiple possible regimens for patients who fail initial therapy.[5–7] The growing experience with treatment of drug-resistant infections may make new regimens in resource-limited settings feasible.[8]

The first-line HAART regimen recommended for low-income and middle-income countries includes a backbone of 2 nucleotide/nucleoside reverse transcriptase inhibitors (NRTIs) with a nonnucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine or efavirenz. The second-line regimen includes 2 similar but not identical NRTIs with a boosted protease inhibitor (bPI) such as lopinavir/ritonavir.[4] Previous studies evaluated the effectiveness and cost-effectiveness of 2-regimen strategies for Africa,[9,10] but to date, no evaluation of strategies employing additional HAART regimens has been done. We evaluated strategies where the current treatment guidelines are expanded to 3 lines of treatment. In addition, we examine the relationship of treatment monitoring to antiretroviral strategies. Recent studies examined the role of monitoring for the current

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2-regimen strategies,[11,12] but the importance of monitoring when using an expanded range of HAART regimens has not been studied. As experience with HAART continues to expand in Africa, additional treatment options are needed. This work will help in planning for the next phase of antiretroviral therapy options in sub-Saharan Africa.

METHODS

Overview

We developed a mathematical simulation model of the clinical course of HIV-infected individuals who present to care in South Africa (see Supplementary Appendix for more details). We compared the effectiveness and costs of 3 alternative HAART strategies: (1) the World Health Organization's (WHO) 2-regimen strategy, (2) a triple-NRTI regimen followed by the WHO regimens, and (3) the WHO regimens followed by a second-generation bPI–based regimen. We only examined regimens that are as easy to administer as the currently available regimens used in sub-Saharan Africa: oral regimens, simple dosing, and little or no requirement for refrigeration (Table 1). We followed each person's health in 1-month increments, though clinical and laboratory data were only available to the patient's clinician during routinely scheduled clinic visits, or sooner for acute events.

Treatment and Monitoring Strategies

We evaluated 3 HAART strategies, 1 with 2 lines of treatment and 2 with 3 lines (Table 1). The 2-line WHO strategy (*a*) included a regimen with 2 NRTIs plus an NNRTI followed by a regimen with a different combination of NRTIs and a bPI.[4] The second strategy (*b*) started with a triple-NRTI regimen followed by 2 regimens similar to the WHO strategy, and the third strategy (*C*) consisted of the WHO sequence followed by a regimen containing a second-generation bPI.

Strategies B and C are realistic in resource-limited settings for clinical and practical reasons. Triple-NRTI regimen hold several advantages as initial therapy in resource-limited regions: they are inexpensive, have relatively low pill burden, avoid important drug interactions, and spare both NNRTIs and PIs, thus maintaining the effectiveness of both drug classes for subsequent regimens. Although initial therapy with triple-NRTI regimens have higher failure rates than initial therapy with NNRTI or bPI, triple-NRTI regimens have been studied in both clinical and cohort trials in Africa.[20,24,25] A third-line regimen with a second-generation bPI is also reasonable, especially after failure of a regimen with first-generation bPI. Easy dosing, side-effect profile comparable to first-generation bPIs, increasing clinical experience, and decreasing costs make this regimen a real possibility for sub-Saharan Africa.[23,26]

The data on rates of failure were taken from cohort and clinical trials (Table 1). Previous estimates of failure rates vary widely based on the clinical setting, definition of failure, existing or new drug resistance mutations, and multiple determinants of adherence, including pill burden, funding source for antiretrovirals, and adherence counseling.[27–31] Most of the available data on treatment failure come from trials which enrolled individuals with HIV subtype B, a rare clade in southern Africa. However, there is no evidence that antiretroviral activity is significantly altered in non–subtype B viruses that predominate in southern Africa.[32] For the base case estimates, we used data from South Africa, followed by data from other sub-Saharan cohort studies, and non-African trials where no other data were available (Table 2). Due to the uncertainty in the estimates, we varied the rates of virologic failure broadly to examine how uncertainty changes the effectiveness and cost-effectiveness of the strategies.

We tested strategies where CD4 counts alone or in combination with viral load were available for patient monitoring. Viral load monitoring is relatively expensive and rarely available in Africa, although there is interest in increasing viral load capacity with the scale-up on HAART as it is the most direct virologic measure of treatment failure.[49]

TABLE 1. Antiretroviral Regimen Strategies

Regimen	Suppressed at 1 yr	Range	Sources
Strategy A: current WHO guidelines			
Regimen 1: 2 NRTIs + NNRTI (based on AZT + 3TC + NVP or EFV)	85	60–90	Boulle et al,[13] Orrell et al,[14] Calmy[15]
Regimen 2: 2 NRTIs + PI/r (based on TDF + 3TC + LPV/r)	70	50–80	De Mendoza et al,[16] Kaufmann et al,[17] Robbins et al[18]
Strategy B: initial triple-NRTI regimen			
Regimen 1: 3 NRTIs (based on AZT + 3TC + ABC)	60	40–70	Staszewski et al,[19] DART,[20] Srikantiah et al[21]
Regimen 2: 2 NRTIs + NNRTI (based on TDF + 3TC + NVP or EFV)	80	50–90	Gulick et al[22]
Regimen 3: 2 NRTIs + PI/r (based on TDF + 3TC + LPV/r)	70	50–80	De Mendoza et al,[16] Kaufmann et al,[17] Robbins et al[18]
Strategy C: third regimen with second-generation PI			
Regimen 1: 2 NRTIs + NNRTI (based on AZT + 3TC + NVP or EFV)	85	60–90	Boulle et al,[13] Orrell et al,[14] Calmy[15]
Regimen 2: 2 NRTIs + PI/r (based on TDF + 3TC + LPV/r)	70	50–80	De Mendoza et al,[16] Kaufmann et al,[17] Robbins et al[18]
Regimen 3: 2 NRTIs + second-generation PI/r (based on TDF + 3TC + DNV/r)	60	30–75	Madruga et al[23]

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; DNV/r, ritonavir-boosted darunavir; EFV, efavirenz; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; PI/r, ritonavir-boosted protease inhibitor; TDF, tenofovir.

TABLE 2. Estimates for Model Variables

Variable	Base Case	Range			Source
Demographic variables					
Age at presentation (mean ± SD)	33 ± 9	—			Holmes et al,[33] Badri et al[34]
Male	60%	50%–70%			Holmes et al,[33] et al,[35] Orrell et al[30]
CD4 at presentation, cells/μL (mean ± SD)	307 ± 227				Holmes et al,[33] Badri et al[34]
Viral load at presentation, log copies/mL (mean ± SD)	5.0 ± 0.8				Badri et al[36]
Disease progression variables					
Decline in CD4 (cells/μL /mo), viral load $>10^5$					Holmes et al,[33] Mellors et al,[37] Rodriguez et al,[38] Ledergerber[39]
Baseline CD4 >500 cells/μL	5.9	±50%			
Baseline CD4 351–500 cells/μL	3.8	±50%			
Baseline CD4 201–350 cells/μL	2.6	±50%			
Baseline CD4 <200 cells/μL	2.0	±50%			
Decline in CD4 (cells/μL/mo), viral load 10^3–10^5					Holmes et al,[33] Mellors et al,[37] Rodriguez et al,[38] Ledergerber[39]
Baseline CD4 >500 cells/μL	3.9	±50%			
Baseline CD4 351–500 cells/μL	2.6	±50%			
Baseline CD4 201–350 cells/μL	1.7	±50%			
Baseline CD4 <200 cells/μL	1.3	±50%			
Monthly probability of developing severe opportunistic diseases (%), by CD4	<50 cells/μL	51–200 cells/μL	201–350 cells/μL	>350 cells/μL	Holmes et al[33]
Oral candidiasis	3.50	2.04	1.26	0.57	
Chronic diarrhea	2.00	0.49	0.18	0.00	
Esophageal candidiasis	1.46	0.34	0.09	0.06	
Wasting syndrome	1.29	0.23	0.02	0.00	
Severe bacterial	1.15	0.04	0.03	0.00	
Pulmonary TB	1.15	0.71	0.47	0.11	
Extrapulmonary TB	0.98	0.47	0.18	0.05	
PCP	0.67	0.05	0.02	0.00	
CMV	0.52	0.07	0.02	0.00	
Cryptococcal meningitis	0.52	0.05	0.00	0.00	
Risk of death, cells/μL					Badri et al[35]
CD4 <50	2.1%/mo				
CD4 51–200	1.7%/mo				
CD4 201–350	1.1%/mo				
CD4 >350	0.8%/mo				
Additional risk of death from severe opportunistic disease, cells/μL					Goldie et al[9]
CD4 <50	7.69%/mo				
CD4 51–200	4.48%/mo				
CD4 201–350	0.66%/mo				
Risk of virologic failure	Table 1				
Harm from discontinuation of a nonsuppressive regimen					Deeks et al[40]
Drop in CD4, cells/μL	128				
Rise in viral load set point, log copies/mL	0.8				
Risk of regimen change or discontinuation due to toxicity, %/mo					
AZT + 3TC + ABC	0.3	0.1–0.5			DART,[20] Munderi and DART[41]
AZT + 3TC + NVP or EFV	0.5	0.3–0.7			Orrell et al,[14] Boulle et al[13]
TDF + 3TC + LPV/r	0.3	0.1–0.5			Amoroso,[42] Calmy[15]
TDF + 3TC + DNV/r	0.5	0.3–0.7			Madruga et al,[23] Clotet et al[26]
Utilization and cost variables					
Annual inpatient cost (US $2007)					Badri et al,[43] Govender et al,[44] Cleary et al,[45] Thomas et al[46]

(*continued on next page*)

TABLE 2. (*continued*) Estimates for Model Variables

Variable	Base Case	Range	Source
No AIDS on HAART	255	230–282	
AIDS on HAART	483	386–596	
No AIDS off HAART	887	839–939	
AIDS off HAART	3632	3304–3985	
Annual outpatient cost			Badri et al,[43] Govender et al,[44] Cleary et al[45]
No AIDS on HAART	316	305–328	
AIDS on HAART	276	248–308	
No AIDS off HAART	158	149–203	
AIDS off HAART	240	207–277	
Annual cost of HAART regimens (US $2007)			Medicines Sans Frontiers,[47,48] Badri et al[43]
3 NRTIs (based on AZT + 3TC + ABC)	548		
2 NRTIs + NNRTI (based on AZT + 3TC + NVP or EFV)	199		
2 NRTIs + PI/r (based on TDF + 3TC + LPV/r)	737		
2 NRTIs + second-generation PI/r (based on TDF + 3TC + DNV/r)	1,332		
Cost of CD4 test (US $2007)	15	10–25	Badri,[43] Zijenah et al[50]
Cost of viral load test (US $2007)	45	15–75	Rouet and Rouzioux,[51] Calmy et al[52]

3TC, lamivudine; ABC, abacavir; AZT, zidovudine; DNV/r, ritonavir-boosted darunavir; EFV, efavirenz; LPV/r, ritonavir-boosted lopinavir; NVP, nevirapine; PI/r, ritonavir-boosted protease inhibitor; TDF, tenofovir.

Failure was defined as 2 successive measurements above 1000 copies per microliter.[14] When CD4 counts only were available, a decline to half the highest measured CD4 count after an initial response was considered failure and prompted a regimen change.[4] Although the WHO currently recommends treatment initiation at 200 cells per microliter, recent evidence and ongoing trials are favoring earlier treatment initiation; thus in our model, treatment was initiated at a CD4 threshold of 200–350 cells per microliter.[53,54]

Disease Progression

The disease model is described in the Supplementary Appendix and elsewhere.[11] The principal determinants of short term mortality were the CD4 counts and development of severe opportunistic diseases.[35] The CD4 counts changed according to HAART regimen, duration of treatment, CD4 at the start of treatment, age, viral load, and opportunistic diseases (Table 2). We modeled the incidence of AIDS-defining opportunistic diseases based on clinical experience in Cape Town and estimated their contribution to mortality, utilization of resources, and costs separately.[33,43]

Costs and Benefits

We included all direct costs of HIV care: inpatient, outpatient, HAART, and monitoring costs. Inpatient and outpatient costs were derived from costing studies in Cape Town, and costs of HAART were taken from published sources and estimated at the lowest available price in US $2007. Second-generation bPI are rarely available in South Africa, and we used expected prices for low-income and middle-income countries.[48] We measured the cost-effectiveness as the ratio of the incremental costs to the incremental benefits of each strategy compared with the next least cost-effective strategy in US $2007 per year of life gained. We adopted a societal perspective, although some indirect costs were excluded as we assumed that they would be equivalent between strategies. We discounted all costs and benefits at 3% annually.

Sensitivity Analysis

In sensitivity analysis, we varied the rates of virologic failure to reflect uncertainty in published estimates. We also varied several important cost parameters that are expected to change. Specifically, bPIs are likely to become less expensive due to entry of additional drugs and continued price negotiations; and viral load monitoring is increasingly affordable due to cheaper technologies, durable measurement devices, and improving infrastructure.[55] We performed a probabilistic sensitivity analysis where we specified distributions for model parameters and employed a Monte Carlo simulation to sample from these distributions. We used the results to calculate confidence intervals (CIs) around our incremental cost-effectiveness ratio estimates.[56] Additional details on the distributions used is in the Supplementary Appendix.

RESULTS

We estimate that over the lifetime of the cohort, 25.6% of individuals would experience virologic failure of WHO's

first-line and second-line regimens. All individuals with virologic failure who remained in care were detected where viral load monitoring was available. However, where CD4 monitoring alone was available, treatment failure based on immunologic criteria was detected in only 6.5% of the cohort. Monitoring individuals with CD4 counts alone led to lower rates of detecting treatment failure primarily due to the insensitivity of immunologic criteria for detecting virologic failure.[57] In addition, monitoring CD4 counts only was associated with a delay of 16.1 months on average in detecting failure. This, in turn, was associated with higher rates of mortality (Table 3) and decreasing the opportunities for detecting treatment failure.

HAART Strategies With CD4 Monitoring

Where CD4 alone was used to monitor treatment success, the base strategy (*a*) was associated with a discounted life expectancy of 78.7 months and lifetime costs of $6299 from the time of presentation to care. In comparison, the strategy with a triple NRTI as initial therapy (*b*) was dominated; that is, it decreased life expectancy and increased costs (Fig. 1). The strategy where a second-generation bPI was used as a third regimen (*c*) was associated with an increase in life expectancy of 18 discounted days and an increase in lifetime costs of $124, an incremental cost effectiveness ratio of $2581 per year of life gained (95% CI 2044 to 3006, using probabilistic sensitivity analysis). Only 6.5% of the population were placed on third-line HAART in strategy C, but the life expectancy of individuals who utilized additional HAART in that strategy was, on average, 8.9 discounted (14.9 undiscounted) months longer than the equivalent population in the WHO strategy (A). The gains in life expectancy were associated with an overall reduction in the incidence of severe opportunistic diseases (Table 3).

HAART Strategies With Viral Load Monitoring

When viral load monitoring was available, the WHO strategy was associated with a discounted life expectancy of 81.0 months and a lifetime cost of $7645. Compared with the WHO strategy, adding a regimen with a second-generation bPI (c) was associated with a gain in life expectancy of 1.6 months and additional $881 in lifetime costs, an incremental cost-effectiveness ratio of $6519 per year of life gained (95% CI 5673 to 8129). The cost of HAART alone was $873 higher in strategy C compared with the WHO strategy. Strategy C was associated with a lower incidence of severe opportunistic diseases, 9.7 per 100 patient-years, compared with 10.9 per 100 patient-years in the WHO strategy. With viral load monitoring, 25.6% of individuals were eligible for third-line treatment under strategy C. The life expectancy of those who were placed on a second-generation bPI was 10.4 undiscounted months longer than the equivalent population in the WHO strategy. Strategy B was dominated by extended dominance. That is, it was more costly and less effective than a blend of strategies A and C but not compared with either strategy alone.

Sensitivity Analyses

Rates of Failure

Rates of failure vary widely based on the HAART regimen, geography, clinical setting (eg,. trial or cohort) and individual treatment history. For that reason, we varied the rates of virologic suppression to reflect a broad range of uncertainty. Decreasing the rates of virologic failure from highest to lowest was associated with an average increase in life expectancy of 5.6 months across all strategies (range 4.9–6.1 months). Decreasing rates of virologic failure were also associated with a less attractive incremental cost-effectiveness ratio of additional HAART regimens. With viral load monitoring, the incremental cost effectiveness ratio of strategy C compared with the WHO strategy varied from $12,098 to $5178 per life-year gained as the rate of virologic failure varied from lowest to highest rate. Thus, the value adding an effective third-line HAART regimen is greatest where rates of failure are relatively high (Fig. 2).

Cost Implications

We varied several cost parameters to examine the implications as drug and diagnostic technologies are increasingly affordable. Specifically, we varied the costs of second-generation bPIs and the cost of viral load testing.

TABLE 3. Lifetime Costs and Outcomes of Alternative Strategies

Strategy*	Discounted Life Expectancy (mo)	Discounted Lifetime Cost ($)	Percent Utilizing Third-Line HAART	Rate of ODs (per 100 Patient-Years)†	Incremental Cost-Effectiveness Ratio ($/Year of Life Gained)
CD4 counts only available					
A	78.7	6299	NA	11.7	—
B	76.1	6892	18.1	12.8	Dominated†
C	79.3	6423	6.5	10.9	2581
Viral loads available					
A	81.0	7645	NA	10.9	—
B	81.1	8006	30.3	11.3	Dominated by extended dominance†
C	82.6	8526	25.6	9.7	6519

ODs, opportunistic diseases.

*The letter refers to the strategies listed in Table 1(A, 2 regimens; B, 3 regimens with triple NRTI as initial therapy; and C, 3 regimens with second-generation PI).

†Dominated strategies had at least 1 other strategy that was more effective and less costly than the dominated strategy. Extended dominance means that some blend of 2 strategies was more effective and less costly than the dominated strategy.

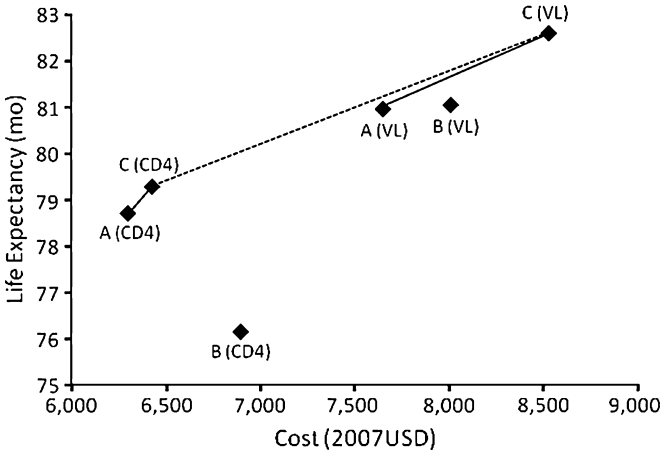

FIGURE 1. Health and cost outcomes of alternative HAART regimens. Life expectancy and lifetime costs of alternative HAART strategies. Letters correspond to strategies in Table 1. (A, 2 regimens; B, 3 regimens with triple NRTI as initial therapy; C, 3 regimens with second-generation bPI). Monitoring strategy is represented as either CD4 counts only (CD4) or CD4 and viral loads (VL). Strategy B is strictly dominated when only CD4 counts are available (it is more costly and less effective than other strategies) and dominated by extended dominance when viral loads are available (it is more costly and less effective than some combination of A and C). Solid lines connect the cost-effective strategies within a given monitoring strategy and the dotted lines connect the cost-effective strategies regardless of monitoring strategy.

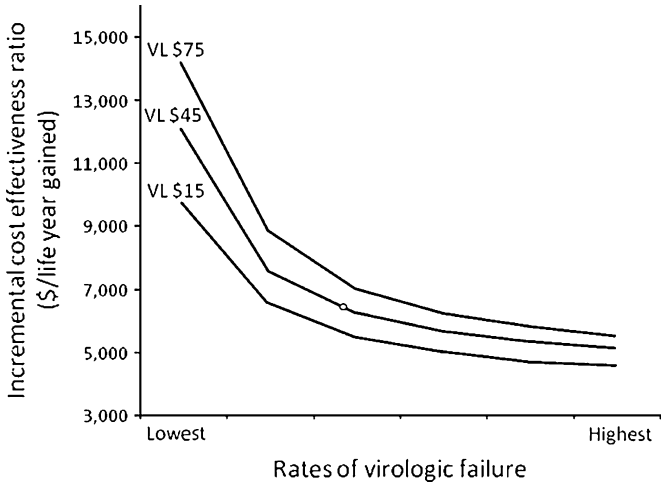

FIGURE 2. Effect of failure rates and viral load cost on cost-effectiveness of a third-HAART regimen. The incremental cost-effectiveness ratio of strategy C compared with the WHO strategy with viral load monitoring as a function of rates of failure and price of viral load monitoring. With lower rates of failure, fewer people require advanced HAART regimens, and the incremental benefits of those regimens diminishes, making the incremental cost-effectiveness ratio higher. The incremental cost-effectiveness ratio depends on the per test cost of viral load monitoring.

When the annual cost of a second-generation bPI dropped below $540, strategy C with CD4 count monitoring dominated the WHO strategy. That is, it saved costs and improved outcomes relative to the WHO strategy. Reducing the cost of viral load monitoring from $75 to $15 per test decreased the incremental cost-effectiveness ratio of adding a second-generation bPI to $5427 per life-year gained. Figure 2 shows the effect of reducing the cost of viral load monitoring on the incremental cost-effectiveness ratio of strategy C compared with the WHO strategy.

Probabilistic Sensitivity Analysis

In probabilistic sensitivity analysis, we examined the joint effect of parameter uncertainty. The analysis was repeated 1000 times, and we used the results to obtain CIs for our estimates. Our 95% confidence bounds for the portion of the population who had virologic failure to WHO's first and second line were 23.4% to 28.8%. In our analysis, the strategy where a triple NRTI was used in initial regimen with CD4 count monitoring was dominated in 88% of the scenarios, we simulated and it never dominated the WHO strategy.

DISCUSSION

We analyzed the benefits, costs, and cost-effectiveness of adding HAART regimens for resource-limited settings using data from South Africa. We show that adding an effective third antiretroviral regimen could provide substantial benefits for those who fail first-line and second-line therapy. Our estimates suggest that individuals who fail both existing regimens may gain between 6.7 and 8.9 months of life with third-line HAART. Although at most a quarter of the infected population could derive benefit from an effective third line, we estimate that that is sufficient to improve the average life expectancy of the entire infected population by 0.6–1.6 months, depending on the monitoring technology. Our estimates of the need for additional regimens, which suggest that the current recommended regimens will provide adequate lifelong benefits for about three quarters of the infected population on HAART, are consistent with recent evidence showing low rates of failure in low-income countries.[58]

The WHO and World Bank suggest that interventions with an incremental cost-effectiveness ratio less than 3 times the gross domestic product (GDP) per capita represent good value.[59,60] By that criteria, adding a third-line regimen based on a second-generation bPI to the existing WHO regimens should be acceptable in South Africa. Although the incremental cost-effectiveness ratio is higher with viral load monitoring (due to higher HAART and monitoring costs), adding a third regimen may be acceptable in countries with an annual per capita GDP above $2000. Further reductions in the price of second-generation bPIs will improve the cost-effectiveness of adding a third regimen and may be cost saving below $540 per year.

Our analysis, however, suggests that adding a less efficacious first-line regimen may worsen outcomes where viral load monitoring is not available. When methods for timing of regimen change are associated with a significant delay, such as when using CD4 counts, adding an initial regimen with rates of failure higher than subsequent regimens may lead to worse

outcomes. Although preserving drug classes has intuitive appeal, the delay in diagnosis of treatment failure without viral load monitoring led to additional opportunistic diseases and higher mortality in our study. Even when viral load monitoring is available, we estimate that the cost-effectiveness of adding an initial triple-NRTI regimen is not as cost effective as adding a second-generation bPI as a third regimen.

We also show the importance of preventing virologic failure in improving patient outcomes. The variability in the rates of failure reported in the literature may be attributed to clinical practices and behavioral factors. Taking HAART regularly is directly related to maintaining virologic suppression, and our study suggests that HAART outcomes improve substantially with lower rates of failure. We find that the relative value of additional HAART regimens is highest where the rates of failure are also high.

Use of CD4 counts to determine when to initiate HAART in resource-limited settings improves life expectancy substantially and may reduce costs.[11] Here we highlight several important roles which viral load monitoring plays. It is the preferred method for timing regimen change: it is more accurate than using CD4 counts for determining treatment failure and leads to a significantly shorter lag in diagnosis and fewer opportunistic diseases. The findings in our analysis dovetail with increasing evidence about the inaccuracy of using CD4 count monitoring alone for determining treatment failure.[57,61] In addition, the benefits of viral load monitoring are greater with more complex treatment options. However, substantial expenditures, lack of infrastructure, and shortage of skilled labor needed for viral load monitoring may continue to be a barrier in many places.

Our model has several important limitations. We estimate rates of virologic failure and medication toxicities from clinical trials and cohort trials. Most of those were done in sub-Saharan Africa on non–HIV subtype B, but where no estimates were available from our region of interest, we used data from developed countries. In addition, available data on the effectiveness of sequential regimens is sparse, and our estimates are partly based on conditional predictions. We also do not account for rates of loss to follow-up, which some suggest may be lower where diagnostic monitoring and medical care is more extensive. Finally, it is also possible that the pathways to resistance of non–HIV subtype B may differ from reported experience. For all these reasons, we vary the rates of failure across a wide range and indicate the limitations of our estimates.

As access to HIV treatment continues to expand across sub-Saharan Africa, where over 20 million are infected and thousands are started on treatment weekly, the number of people who will fail the existing regimens will continue to increase. We suggest that offering additional effective regimens provide substantial benefits to individuals who fail existing therapies, is cost effective in many parts of southern Africa where CD4 count monitoring is available, and may be cost saving with substantial price reductions of second-generation bPIs. Our analysis also shows that reducing treatment failure is an effective way to minimize the need for additional regimens and maximize the benefits of the regimens that are currently available.

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Scaling Up the 2010 World Health Organization HIV Treatment Guidelines in Resource-Limited Settings: A Model-Based Analysis

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Abstract

Background: The new 2010 World Health Organization (WHO) HIV treatment guidelines recommend earlier antiretroviral therapy (ART) initiation (CD4<350 cells/μl instead of CD4<200 cells/μl), multiple sequential ART regimens, and replacement of first-line stavudine with tenofovir. This paper considers what to do first in resource-limited settings where immediate implementation of all of the WHO recommendations is not feasible.

Methods and Findings: We use a mathematical model and local input data to project clinical and economic outcomes in a South African HIV-infected cohort (mean age = 32.8 y, mean CD4 = 375/μl). For the reference strategy, we assume that all patients initiate stavudine-based ART with WHO stage III/IV disease and receive one line of ART (stavudine/WHO/one-line). We rank—in survival, cost-effectiveness, and equity terms—all 12 possible combinations of the following: (1) stavudine replacement with tenofovir, (2) ART initiation (by WHO stage, CD4<200 cells/μl, or CD4<350 cells/μl), and (3) one or two regimens, or lines, of available ART. Projected life expectancy for the reference strategy is 99.0 mo. Considering each of the guideline components separately, 5-y survival is maximized with ART initiation at CD4<350 cells/μl (stavudine/<350/μl/one-line, 87% survival) compared with stavudine/WHO/two-lines (66%) and tenofovir/WHO/one-line (66%). The greatest life expectancies are achieved via the following stepwise programmatic additions: stavudine/<350/μl/one-line (124.3 mo), stavudine/<350/μl/two-lines (177.6 mo), and tenofovir/<350/μl/two-lines (193.6 mo). Three program combinations are economically efficient: stavudine/<350/μl/one-line (cost-effectiveness ratio, US$610/years of life saved [YLS]), tenofovir/<350/μl/one-line (US$1,140/YLS), and tenofovir/<350/μl/two-lines (US$2,370/YLS).

Conclusions: In settings where immediate implementation of all of the new WHO treatment guidelines is not feasible, ART initiation at CD4<350 cells/μl provides the greatest short- and long-term survival advantage and is highly cost-effective.

Please see later in the article for the Editors' Summary.

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Abbreviations: ART, antiretroviral therapy; CEPAC, Cost Effectiveness of AIDS Complications; SD, standard deviation; WHO, World Health Organization; YLS, years of life saved

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Introduction

The 2006 World Health Organization (WHO) guidelines on antiretroviral therapy (ART) established a worldwide standard of care for patients with HIV infection [1]. Since this publication, new evidence has emerged on how to treat patients infected with HIV, and this evidence formed the basis for the WHO 2010 ART guidelines update [2]. These revisions aim to better align global standards with those already adopted in well-resourced countries [3,4]. Specifically, revised guidelines recommend an increased number of sequential ART regimens, routinely available CD4 count monitoring, earlier ART initiation thresholds (CD4<350 cells/μl versus CD4<200 cells/μl), and replacement of stavudine with the less-toxic drug tenofovir.

As WHO expands treatment recommendations, many countries in resource-limited settings still struggle to implement 2006 guidelines [5]. In Malawi, for example, most HIV disease is monitored clinically; CD4 count monitoring is limited to pregnant women and children [6,7]. In South Africa, ART is available to only 22%–36% of those reported to be in need [8]. In settings confronted with numerous new recommendations, not all of which are immediately feasible, the relevant policy question is: *What to do first?* Should countries begin by replacing stavudine with tenofovir or by making CD4 count monitoring universally available? To assist policy makers in this prioritization process, we use a model-based analysis with data from South Africa to project the clinical and economic outcomes of alternative stepwise implementation scenarios toward the 2010 WHO ART guidelines.

Methods

Analytic Overview

The Cost Effectiveness of AIDS Complications (CEPAC)–International model is a Monte Carlo simulation model of the natural history and treatment of HIV disease (see Text S1 for model details) [9–11]. We populate the model with South African clinical and resource utilization data to project survival and costs under alternative guideline prioritization scenarios. We use a "no ART" scenario for comparison and assume that baseline care (designated the "reference strategy") is a one-line stavudine-containing regimen, initiated at WHO stage III or IV disease, without CD4 count monitoring capacity. We then examine every feasible sequence of the following implementation elements: (1) widespread CD4 count monitoring capacity, allowing for ART initiation at CD4<200 cells/μl (and biannual monitoring), (2) earlier ART initiation, at CD4<350 cells/μl (assumes CD4 count availability), (3) an available second-line ART regimen upon first-line failure, and (4) replacement of stavudine with tenofovir in the first-line regimen. To refer to these strategies, we use the following nomenclature: nucleoside analog in first line/ART initiation criterion/number of regimens [e.g., stavudine/<200/μl/two-lines]). The combined implementation elements result in twelve possible strategies, in addition to no ART (Figures 1 and S1). We examine the short- and long-term survival benefits and cost-effectiveness of each stepwise, incremental policy change from the reference strategy to full 2010 guideline implementation. We also evaluate the cost and survival impact of imposing an additional "equity" constraint—i.e., that all members of the cohort at any given time are provided the same treatment program. Finally, we use sensitivity analyses to examine the efficacy and cost input parameters necessary to change the conclusions.

When reporting clinical outcomes alone (per-person life expectancy), we provide undiscounted results. When clinical and economic results are used to create cost-effectiveness ratios, we adhere to established convention in discounting both at 3% per annum [12]; cost-effectiveness ratios are reported in US dollars per year of life saved (dollars/YLS) (See Text S1 for details). We conduct an "incremental" assessment of economic costs and health benefits, as recommended by the US Panel on Cost-Effectiveness in Health and Medicine [12]. Cost and health outcomes are estimated for all 12 strategies (as well as no ART). These are then ranked in order of increasing cost. After eliminating all "dominated" strategies (i.e., strategies that both cost more and confer fewer benefits than any combination of other strategies), we compute the ratio of incremental costs to incremental benefits for each strategy, comparing it to its next-least-costly, non-dominated alternative [12].

Costs are converted to 2008 US dollars using the South African gross domestic product deflators and the 2008 mean exchange rate between the South African rand and the US dollar (8.23 rand = US$1) [13,14]. Guided by the recommendations of the WHO Commission on Macroeconomics and Health, we consider interventions to be cost-effective in a given country if their cost-effectiveness ratio is less than 3 times the national per capita gross domestic product (South African 2008 gross domestic product = US$5,700) [14].

The CEPAC-International Model

The CEPAC-International model simulates the progression of disease in a hypothetical cohort of patients infected with HIV as a sequence of monthly transitions between health states. Health states are defined to be clinically and economically representative of the disease course and are stratified by current CD4 count, current HIV RNA level, and history of opportunistic disease. A graphical representation of a patient trace in South Africa is presented in Figure S2, illustrating CD4 cell count, HIV RNA, and clinical events, including tuberculosis, over a hypothetical patient's lifetime. We are careful to distinguish in the model "actual" CD4 cell count and HIV RNA—i.e., the underlying immunologic and virologic state, regardless of whether they are measured by a laboratory test—from "observed" CD4 cell count and HIV RNA—that which is measured by a test and upon which clinical decisions can be made. Actual CD4 cell count determines the frequency of opportunistic diseases, while ART influences actual HIV RNA levels and CD4 cell counts. Health states therefore reflect the underlying disease process, and clinical decisions (ART initiation or switch) are based on observed factors such as presentation with an opportunistic disease, or CD4 count, if monitoring is available. Reflecting standards of care in most sub-Saharan African nations, HIV RNA monitoring is assumed to be unavailable [1]. Patients are followed from entry into HIV care through death.

In strategies without available CD4 monitoring, decisions regarding ART initiation and switching are made based upon observation of any of the following severe opportunistic diseases representative of WHO stage III/IV disease: severe bacterial infection, severe fungal infection, tuberculosis, toxoplasmosis, nontuberculous mycobacteriosis, *Pneumocystis jiroveci* pneumonia, or other WHO stage IV defining diseases. Two mild opportunistic diseases (fungal and other) result in resource utilization but no changes in the ART decision-making process. Patients die in the model from an acute event (e.g., an opportunistic disease or a drug-related toxicity), from chronic HIV disease, or at South African age- and sex-adjusted background mortality rates [15].

The frequency of clinical and laboratory assessments in the model is user-defined. For this analysis, we have chosen clinical assessments to occur every 3 mo; in strategies where CD4 counts are available, they are modeled as being performed biannually.

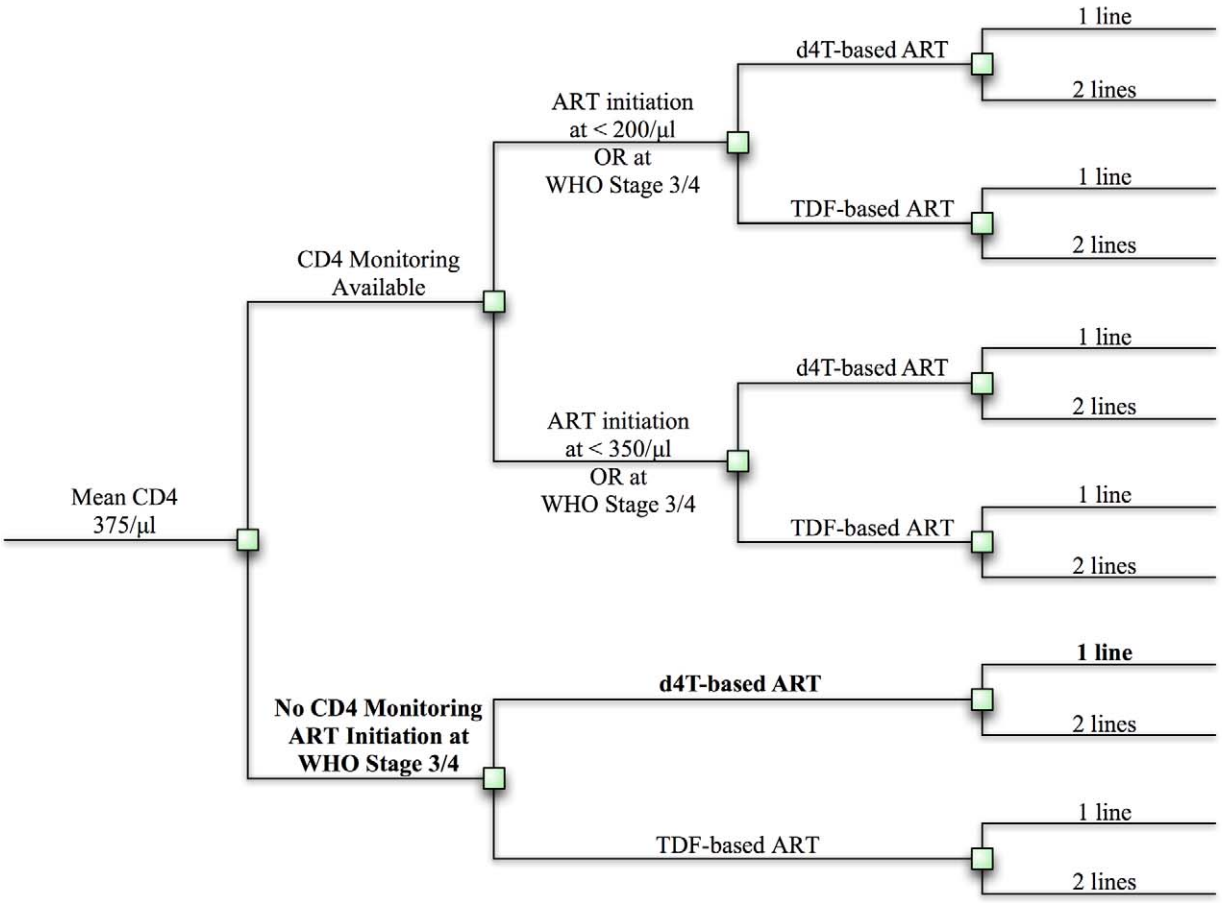

Figure 1. Clinical and policy decisions yield 12 implementation strategies. Clinical and policy decisions result in 12 possible implementation strategies. These strategies are listed in Text S1. Squares represent decision points. The reference strategy is bolded. d4t, stavudine; TDF, tenofovir.
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ART is initiated when one of two criteria is met: falling below a defined CD4 count threshold or the development of WHO III/IV disease (i.e., severe opportunistic disease). Effective ART in the model results in actual virologic suppression (independent of gender), a CD4 count increase, and a CD4-independent reduction in risk of opportunistic diseases and chronic AIDS death [16–19]. Because HIV RNA monitoring is unavailable, virologic failure on an antiretroviral regimen is itself not detected. However, the impact of virologic failure is ultimately observed when it manifests with immunologic dysfunction through either a documented opportunistic disease or a CD4 decline that is revealed by laboratory testing. Six months after ART initiation, observed treatment failure is defined as meeting any one of the following three criteria: the development of a severe opportunistic disease, observation of a 50% decline from peak on-treatment CD4 count, or observation of two consecutive CD4 counts below 100 cells/µl [1]. Upon observed treatment failure, ART is switched if a subsequent regimen is available or, if not, the failed regimen is continued until death to maintain its modest decreases in the rates of opportunistic disease and death [17,18]. For the purposes of this analysis, we assume no treatment interruptions.

Stavudine- or tenofovir-related toxicity occurs with a one-time probability, distributed over time since drug initiation. Depending on the nature of the toxicity, toxicity results in a one-time cost and/or a duration of costs spanning the time of increased need for care. Certain types of toxicity—including lactic acidosis, lipodystropy, neuropathy, and nephrotoxicity—also result in a single drug switch to zidovudine.

Evaluating Uncertainty

To converge on stable model output, we run a simulated cohort of 1 million patients infected with HIV. Because the cohort size can be varied in the simulation—i.e., we might also simulate 2 million or 5 million patients—95% confidence intervals and standard deviations (SDs) do not adequately capture uncertainty in simulation modeling. Instead, we adhere to the guidance of the US Panel on Cost-Effectiveness in Health and Medicine for reporting uncertainty in deterministic methods [12]. We use univariate sensitivity analysis to examine the impact of variation in individual input parameters. Having identified those variables that exert the greatest influence on our conclusions, we then turn to multivariate sensitivity analyses to examine the impact of simultaneous variation in multiple parameters. This approach results in a large variety of univariate and multivariate sensitivity analyses. We report those instances in which variation of an underlying parameter value has material impact on the findings and conclusions. A more comprehensive description of relevant sensitivity analyses is provided in Texts S2 and S3.

Input Parameters

Data sources for individual input parameters are referenced in Table 1 and in Text S1.

Cohort characteristics. We define an ART-naïve cohort of patients with HIV in South Africa, with mean age 32.8 y [20]. We intentionally choose an initial cohort with a relatively high mean CD4 cell count of 375 cells/μl (SD, 25 cells/μl). A cohort with a lower mean CD4 cell count would not clearly demonstrate the benefits of an ART initiation threshold of CD4<350 cells/μl, as illustrated in sensitivity analyses (Text S2). Over 40% of the cohort has HIV RNA>100,000 copies/ml (Table 1) [21]. In the model, this ART-naïve cohort is then subject to the policies of ART initiation and drug availability as indicated by each of the 12 strategies. In the absence of ART, the model tracks the patients' natural history of disease for use in comparing the incremental clinical benefits and costs. Figure S3 illustrates the internal validation of South African data used to derive critical model input parameters such as monthly mortality and opportunistic disease incidence rates, stratified by CD4 count.

Opportunistic disease prophylaxis and ART efficacy. All patients at model entry are provided co-trimoxazole prophylaxis, conferring protection against mild and severe bacterial infections, *P. jirovecį* and toxoplasmosis [22,23]. We assume a non-nucleoside reverse transcriptase inhibitor–based ART regimen that includes stavudine. This regimen results in a 24-wk virologic suppression rate of 75% with a mean 48-wk CD4 count rise of 136 cells/μl among those with suppression [19]. The monthly probability of virologic failure after 24 wk is 0.02. When stavudine is replaced with tenofovir in first-line regimens, in the absence of reliable efficacy data for a tenofovir-based regimen in resource-limited settings, we use a virologic suppression rate of 85% at 24 wk, as reported in clinical trials [24,25]. Despite the improved rates of virologic suppression, we want to maintain conservative assumptions with regard to CD4 benefit among those suppressed, so we use the same benefit (136 cells/μl) as that used for the stavudine-based regimen [19]. From these studies, the monthly probability of failure of tenofovir-based ART after 24 wk is 0.01 [24,25].

When second-line ART is available, it is a lopinavir/ritonavir-based regimen with a 24-wk suppressive efficacy of 78%, a resultant CD4 count increase of 151 cells/μl, and a 0.03 monthly probability of virologic failure after 24 wk [16]. In sensitivity analyses, we examine the impact of improved efficacy of first-line ART associated with the use of tenofovir and the impact of alternative second-line ART efficacies (Text S3).

Costs. We consider HIV-associated direct medical costs, including inpatient days, outpatient visits, medication costs, and laboratory tests, when available (Table 1). Direct non-medical costs and indirect costs are excluded. Costs attributable to inpatient hospitalization resulting from an opportunistic infection are calculated as the mean cost of each inpatient day multiplied by the mean length of stay for any given opportunistic disease. Outpatient care costs include the mean cost of each visit, inclusive of standard laboratory tests and procedures. Routine care costs are stratified by CD4 cell count to account for the increased frequency of visits that may be attributable to lower CD4 cell counts (Table 1). The stavudine-based first-line regimen costs US$100 per person-year (stavudine component = US$36), and the tenofovir-based regimen costs US$204 per person-year (tenofovir component = US$135) [26]; all other first-line regimen costs are identical. Second-line ART regimens, when available, cost US$669 per person-year [26]; CD4 count tests cost US$25 each [27,28]. Tenofovir, second-line ART, and CD4 monitoring costs are each varied in sensitivity analyses.

Results

Prioritization by Survival Benefits (Undiscounted)

An untreated HIV-infected South African cohort starting with a mean CD4 count of 375 cells/μl (SD, 25 cells/μl) has a mean undiscounted life expectancy of 47.9 mo. A single-line stavudine-based ART regimen, initiated on development of WHO stage III/IV disease ("reference strategy"; stavudine/WHO/one-line) increases life expectancy to 99.0 mo. Table 2 provides the projected 5-y survival and life expectancies of alternative stepwise progressions toward the 2010 WHO recommendations. Compared to stavudine/WHO/one-line (step 1), 5-y survival is largest (87% survival) with the addition of CD4 count availability and ART initiation at CD4<350 cells/μl (stavudine/<350/μl/one-line). In this initial step, tenofovir/WHO/one-line (66%), stavudine/<200/μl/one-line (80%), or stavudine/WHO/two-lines (66%) each yield lower projected short-term survival. Considering each of the guideline components, stavudine/<350/μl/one-line also produces the greatest anticipated life expectancy increase, Δ25.3 mo. With stavudine/<350/μl/one-line (step 2), adding a second-line regimen results in the next largest life expectancy increase (stavudine/<350/μl/two-lines, Δ53.3 mo). The final step replaces stavudine with tenofovir (tenofovir/<350/μl/two-lines, Δ16.0 mo, step 3), resulting in a comprehensive strategy concordant with the 2010 WHO guidelines, a 5-y survival of 91%, and a projected life expectancy of 193.6 mo (Table 2).

Model-generated survival curves are provided for no ART, the reference strategy, and the three steps in Table 2, which act stepwise to maximize life expectancy (Figure 2). Marked differences in early survival are attributable to earlier ART initiation thresholds; differences in survival later in the disease course are associated with second-line ART availability.

Prioritization by Cost-Effectiveness

Incremental cost-effectiveness analysis (Table 3) reveals three non-dominated strategies (i.e., strategies that attain a given survival level by the least costly means): (1) stavudine/<350/μl/one-line (US$610/YLS), (2) tenofovir/<350/μl/one-line (US$1,140/YLS), and (3) tenofovir/<350/μl/two-lines (US$2,370/YLS). All other strategies are "dominated"—i.e., they are more expensive and confer less survival benefit than some other combination of strategies. Figure 3 (upper panel) maps the 13 strategies on a discounted cost and life expectancy plane. The line connecting the non-dominated strategies designates the "efficient frontier," which represents both the least expensive way to attain a given survival and the maximum achievable survival for any given cost [12].

Thus, a country with a current stavudine/WHO/one-line policy (Figure 3, lower panel) could switch to a tenofovir/<350/μl/one-line policy (open arrow) and thereby simultaneously decrease projected per-person lifetime costs and improve survival. Similarly, a country with a stavudine/<200/μl/one-line policy could decrease per-person costs and also improve outcomes by changing to a stavudine/<350/μl/one-line policy (solid arrowhead). Countries with a stavudine/<200/μl/two-lines policy would require increased per-person expenditures to achieve the survival benefits associated with tenofovir/<350/μl/two-lines, as suggested in the revised WHO guidelines (dotted arrow).

Evaluating the Cost of Equity

Of the three efficient programs (Table 3; Figure 3), tenofovir/<350/μl/one-line has a projected per-person lifetime cost of US$6,870, and tenofovir/<350/μl/two-lines has a projected lifetime cost of US$12,820. An HIV program budget that allows for a per-person cost between US$6,870 and US$12,820 might be

Table 1. Model input parameters for analysis of the 2010 WHO ART guidelines.

Variable	Estimate	Reference
Initial cohort characteristics		
Age, mean years ± SD	32.8±9.2	[20]
Male (%)	54.6	[20]
Distribution of initial CD4, mean cells/μl (SD)	375 (25)	Assumption
HIV RNA distribution (%)		[21]
>100,000 copies/ml	42.5	
30,001–100,000 copies/ml	28.3	
10,001–30,000 copies/ml	17.9	
3,001–10,000 copies/ml	7.8	
501–3,000 copies/ml	2.3	
<500 copies/ml	1.2	
Natural history of disease		
Mean monthly CD4 decline (cells/μl) by HIV RNA stratum (copies/ml)		[35]
>30,000	6.4	
10,001–30,000	5.4	
3,001–10,000	4.6	
501–3,000	3.7	
Monthly risk of severe opportunistic infections (%)[a]		[20]
Bacterial	0.08–0.71	
Fungal	0.02–2.22	
Tuberculosis	0.21–1.96	
Toxoplasmosis	0.00–0.06	
Nontuberculosis mycobacteriosis	0.00–0.30	
P. jiroveci pneumonia	0.00–0.12	
Other severe opportunistic infections	0.25–2.57	
Monthly risk of mild opportunistic diseases (%)[a]		[20]
Fungal	0.59–3.51	
Other	2.51–3.10	
Efficacy of co-trimoxazole (% reduction in probability of infection)		
Severe bacteria	49.8	[22,23]
Mild fungal infections	−46.4[b]	[22,23]
Toxoplasmosis	83.2	[22,23]
P. jiroveci pneumonia	97.3	[22,23]
Other WHO stage IV defining diseases	17.9	[22]
Efficacy of ART (range examined)		
First line: stavudine-based regimen		[19]
HIV RNA suppression	75% at 24 wk	
CD4 count increase	136 cells/μl at 48 wk	
Probability of later failure (monthly, after 24 wk)	0.02[c] (0.01–0.02)	
First line: tenofovir-based regimen		
HIV RNA suppression	85% at 24 wk (85%–95%)	[24]
CD4 count increase	136 cells/μl at 48 wk	[19]
Probability of later failure (monthly, after 24 wk)	0.01[d] (0.005–0.01)	[24]
Second line: lopinavir/ritonavir-based regimen		[16]
HIV RNA suppression	78% at 24 wk (40%–88%)	
CD4 count increase	151 cells/μl at 48 wk	
Probability of later failure (monthly, after 24 wk)	0.03[e] (0.01–0.06)	
Toxicity (one-time probability [%])		
Stavudine-based regimen (range examined)		
Severe lactic acidosis	1.7 (1.7–3.4)	[36]

Table 1. Cont.

Variable	Estimate	Reference
Lipodystrophy	1.3 (1.3–2.6)	[36]
Neuropathy	2.6 (2.6–5.2)	[36]
Tenofovir-based regimen (range examined)		
Nephrotoxicity	1.6 (1.6–3.2)	[37,38]
Anemia	0.4 (0.4–0.8)	[24]
Discount rate	3%	[12]
Costs (2008 US dollars) (range examined)		
Co-trimoxazole prophylaxis (monthly)	1.03	[23]
Stavudine-based first-line ART (monthly)	8.33	[26]
Tenofovir-based first-line ART (monthly)	17.00 (10.00–17.00)	[26]
Lopinavir/ritonavir second-line ART (monthly)	55.75 (8.36–55.75)	[26]
Routine care (range by CD4, monthly)[a]	9.99–131.23	[20,39,40]
Inpatient hospital care, per day	224.25	[39]
Outpatient hospital care, per visit	10.87	[39]
CD4 count test	US$25 (25–75)	[27,28,41]

"Range examined" indicates that we examined both extreme and intermediate values within the specified ranges.
[a]Range indicated by CD4 count; details by CD4 strata are presented in the Text S1.
[b]The percent monthly risk of mild fungal infections is increased by 46.4% in the presence of co-trimoxazole [22].
[c]Projected using published 24-wk data [19].
[d]Estimated from published 24- and 48-wk data [24].
[e]Estimated from published 24- and 48-wk data [16].
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achieved in several ways; two are illustrative. The first would be to proportionately divide the cohort between two of the programs along the efficient frontier, so that part of the cohort receives tenofovir/<350/µl/one-line and the rest receives tenofovir/<350/µl/two-lines. An alternative would be to provide everyone in the cohort a third program—one that lies below the efficient frontier. The opportunity cost (e.g., the anticipated net loss in discounted life expectancy associated with an alternative strategy choice) of any non-efficient strategy may be quantified by measuring its vertical distance from the efficient frontier. To illustrate this opportunity cost, we take an arbitrary affordability threshold of US$11,500 per person. In the example of a program that can afford no more than US$11,500 per person (stavudine/<200/µl/two-lines; Figure 3, lower panel), the opportunity cost of uniformity in care ("equity") is 14.5 mo per person of survival (shown by the bracket in Figure 3, lower panel).

Sensitivity Analyses

Clinical parameters. In sensitivity analyses, we examine changes in clinical input data required to alter the stepwise

Table 2. Projected life expectancies associated with alternative choices in the stepwise progression toward full implementation of the 2010 WHO HIV treatment guidelines.

Step	5-y Survival (%)	Projected Life Expectancy (Months)	Δ Projected Life Expectancy (months)[a]
Step 1: begin with stavudine/WHO/one-line (reference strategy) (four options)	65	99.0	—
(1) Switch from stavudine to tenofovir, or	66	112.9	13.9
(2) Add CD4 monitoring capacity, initiate ART at CD4<200 cells/µl, or	80	115.6	16.6
(3) Add second-line ART regimen, or	66	121.4	22.4
(4) Add CD4 monitoring capacity, initiate ART at CD4<350 cells/µl	**87**	**124.3**	**25.3**
Step 2: begin with stavudine/<350/µl/one-line (two options)	87	124.3	—
(1) Switch from stavudine to tenofovir, or	89	144.8	20.5
(2) Add second-line ART regimen	**91**	**177.6**	**53.3**
Step 3: begin with stavudine/<350/µl/two-lines (one remaining option)	91	177.6	—
(1) Switch from stavudine to tenofovir	**91**	**193.6**	**16.0**

We use the following nomenclature to define the strategies: nucleoside analog used in first line/ART initiation criterion/number of available regimens. All strategies with initiation criteria indicated by a CD4 count threshold assume availability of CD4 count monitoring. For each step, the option that maximizes survival is shown in bold.
[a]Change relative to the program selected in the previous step.
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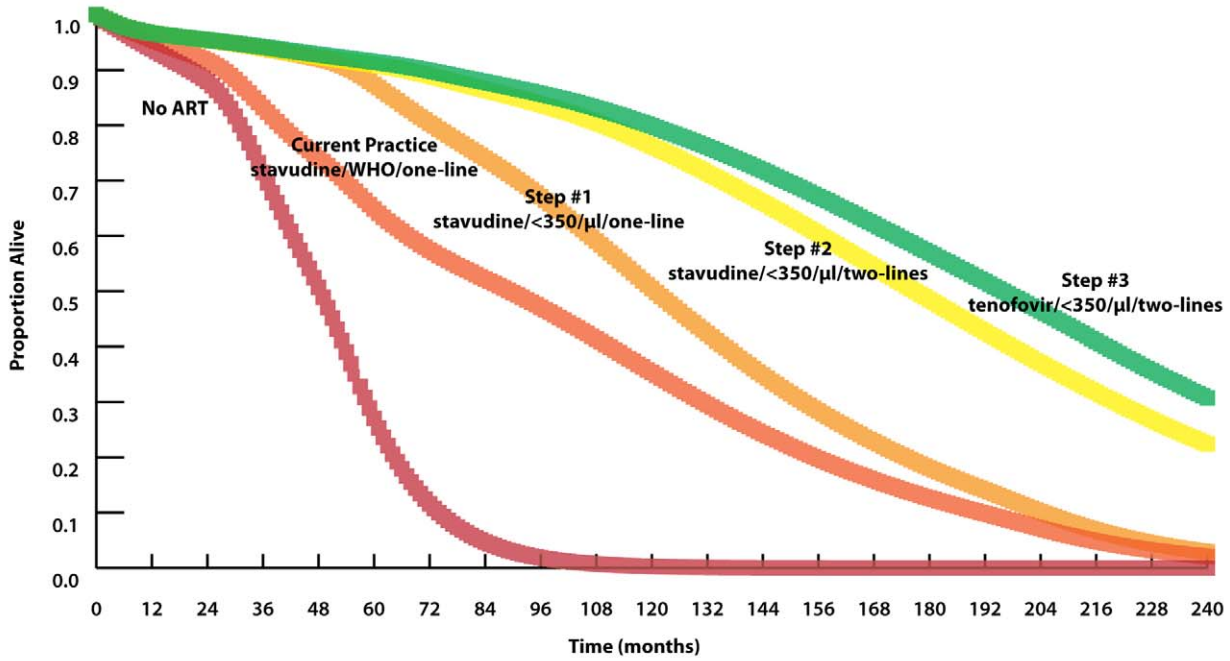

Figure 2. Model-projected survival curves. Model-projected survival curves (undiscounted) of the reference strategy (stavudine/WHO/one-line) and the three strategies projected to maximize life expectancy in stepwise progression toward the 2010 WHO guidelines (see Results and Table 2 for details). Curves highlighting outcomes over the next 5 y are provided in Figure S4. The 20-y horizon is presented here, not to imply that HIV treatment will remain unchanged over this time horizon, but rather to demonstrate when different interventions will have meaningful survival impacts. Median survival increases from 90 mo with stavudine/WHO/one-line (reference strategy) to 121 mo with the addition of CD4 monitoring and ART initiation at CD4<350 cells/µl (stavudine/<350/µl/one-line, step 1) to 177 mo with the addition of a second-line ART regimen (stavudine/<350/µl/two-lines, step 2). A subsequent switch from stavudine to tenofovir results in a comparatively modest survival advantage, with a median survival increase to 196 mo (tenofovir/<350/µl/two-lines, step 3). The survival curve of step 3 represents what might be expected when all the 2010 WHO treatment guidelines are fully implemented.
doi:10.1371/journal.pmed.1000382.g002

ordering of program additions. Modest reductions in the mean CD4 count of the cohort (to 250 cells/µl) show decreased clinical benefits to earlier ART initiation but no substantial changes in cost-effectiveness. When the mean CD4 count of the cohort is less than 100 cells/µl, the benefits of a policy change to earlier ART initiation are largely irrelevant (Text S2). This is because the majority of the cohort is already ART-eligible regardless of the initiation criterion (WHO stage III/IV disease, CD4<200 cells/µl, or CD4<350 cells/µl). Although CD4 monitoring still improves cohort survival compared to clinically based ART initiation, in populations with mean CD4 counts far below the policy-relevant ART initiation criteria, the addition of a second-line regimen becomes the most clinically beneficial intervention. For the anticipated life expectancy benefits of tenofovir/WHO/one-line to exceed those expected with stavudine/<350/µl/one-line, replacement of stavudine with tenofovir would have to increase the 24-wk suppressive efficacy from 85% to 95% and simultaneously decrease the monthly probability of later virologic failure by 50% (from 0.01 to 0.005) (Text S3) [24]. Second-line ART maintains its position in the stepwise order (step 2) as long as its 24-wk viral suppression rate remains between 40% and 88%, even with a 3-fold increase in the rate of late failure when efficacy decreased to 40% (Text S3). Increasing stavudine toxicity by 2-fold alters life expectancy estimates by less than 1 mo and does not change the recommended stepwise additions (Text S3). Similarly, changes in the gender distribution of the cohort have little impact on the results (Text S3).

Cost parameters. Holding efficacy constant, results are very sensitive to the price of tenofovir; a decrease in the cost of tenofovir from US$135 to US$51 per person per year would make tenofovir both more effective and less costly than stavudine. Results are less sensitive to the costs of second-line regimens (15% of base case) and CD4 monitoring (three times base case), neither of which produced meaningful changes in cost-effectiveness results (Text S2). In two-way sensitivity analyses, where the cost of tenofovir is decreased and its efficacy increased, tenofovir/<350/µl/one-line dominates stavudine/<350/µl/one-line when the tenofovir regimen costs are US$153 annually (75% of the base case) and its 24-wk suppressive efficacy is 90% (5% increase from the base case).

Additional sensitivity analyses. Further sensitivity analyses are detailed in the Texts S2 and S3. In Text S2, we present the 1- through 5-y survival rates for all 12 strategies examined, as well as the survival curves of the stepwise strategies selected on a 5-y, rather than a 10-y, horizon (Figure S4). Text S2 also provides the details of analyses under conditions of alternative mean CD4 counts for the cohort and alternative costs of both second-line regimens and CD4 monitoring. Further analyses (Text S3) offer

Table 3. Life expectancy, costs, and incremental cost-effectiveness ratios of the 12 possible stepwise combinations (and no ART) from the reference strategy to full implementation of 2010 WHO HIV treatment guidelines.

Strategy[a]	Discounted Cost	Discounted Per-Person Life Expectancy (Undiscounted) Months	Incremental Cost-Effectiveness Ratio (US$/YLS)
No ART	2,540	44.9 (47.9)	
Stavudine/<350/μl/one-line (step 1)	**5,550**	**104.3 (124.3)**	**610**
Stavudine/<200/μl/one-line	5,740	97.3 (115.6)	Dominated[b]
Tenofovir/<350/μl/one-line	6,870	118.3 (144.8)	1,140
Tenofovir/<200/μl/one-line	6,930	109.9 (133.9)	Dominated[b]
Stavudine/WHO/one-line (reference strategy)	**7,440**	**84.5 (99.0)**	**Dominated[b]**
Tenofovir/WHO/one-line	8,400	93.9 (112.9)	Dominated[b]
Stavudine/WHO/two-lines	10,140	98.8 (121.4)	Dominated[b]
Tenofovir/WHO/two-lines	10,640	105.0 (131.2)	Dominated[b]
Stavudine/<200/μl/two-lines	11,460	127.0 (161.3)	Dominated[c]
Tenofovir/<200/μl/two-lines	11,930	135.3 (175.5)	Dominated[c]
Stavudine/<350/μl/two-lines (step 2)	**12,270**	**138.7 (177.6)**	**Dominated[c]**
Tenofovir/<350/μl/two-lines (step 3)	**12,820**	**148.4 (193.6)**	**2,370**

The reference strategy and the strategies selected in the stepwise progression in Table 2 are shown in bold.

[a]We use the following nomenclature to define the strategies: nucleoside analog used in first line/ART initiation criterion/number of available regimens. All strategies with initiation criteria indicated by a CD4 count threshold assume availability of CD4 count monitoring; WHO indicates WHO stage III/IV disease.

[b]Strongly dominated (more expensive but confer less clinical benefit than some other strategy) [12].

[c]Weakly dominated (more expensive but confer less clinical benefit than some combination of other strategies) [12].

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additional comprehensive analytic variations in cohort gender distributions, ART initiation criteria, first- and second-line ART efficacies, stavudine-related toxicities, and costs. Within plausible ranges, these sensitivity analyses, other than those reported above, had little impact on clinical- or policy-relevant results.

Discussion

The new 2010 WHO ART guidelines aim to promote public health interventions that "secure the greatest likelihood of survival and quality of life for the greatest number" of individuals with HIV. The reported guiding principles in the revision process include: (1) do no harm, (2) ensure access and equity, (3) promote quality and efficiency, and (4) ensure sustainability. Motivated by these tenets, the new guidelines recommend a single CD4-based ART initiation criterion for all populations, a switch from stavudine to tenofovir, and universally available second-line regimens [2]. We find that in settings where immediate implementation of all of the new WHO treatment guidelines is currently not feasible, ART initiation at CD4<350 cells/μl provides the greatest short- and long-term survival advantage and is very cost-effective. In countries that are already initiating stavudine at CD4<350 cells/μl and have access to CD4 monitoring, switching from stavudine to tenofovir increases survival and is also cost-effective. Access to second-line ART provides more clinical benefit than access to tenofovir but at substantially greater costs.

The additional outlays implied by the new guidelines stand in stark contrast to the resource-constrained reality encountered on the ground. Many countries are still striving to meet goals set by the now-superseded 2006 guidelines. The WHO estimates the current ART coverage rate across low- and middle-income countries to be 42% [5,29]. Meanwhile, the new guidelines recommend access to CD4 count monitoring, call for treatment of almost double the 3–5 million people already requiring treatment based on the previous guidelines [30], and suggest replacement of the most widely used antiretroviral drug with one that costs nearly US$100 per patient-year more [26]. In most resource-limited settings, the relevant policy questions are: *What is feasible now?* and *What to do first?*

Based on projected short- and long-term survival and cost-effectiveness results, we identify three critical messages. First, countries with very limited resources and still only one line of ART available should focus first on access to CD4 count monitoring and ART initiation at CD4<350 cells/μl. These should be implemented before switching from stavudine to tenofovir and prior to providing second-line ART. Although advising to use stavudine in the first-line regimen—with its inherent toxicities—may be seen as conflicting with the primary WHO principle "first, do no harm," the switch from stavudine to tenofovir is the recommendation that provides the least overall increase in survival, according to the results presented here. Initiating stavudine-based ART at CD4<350 cells/μl, compared with clinically based ART initiation, provides immediate and substantial short-term survival benefits, yields the greatest life expectancy compared to other guideline components, and is cost-effective by international standards. In cases where most patients present to care with CD4 counts far below the ART initiation threshold (e.g., CD4<100 cells/μl), a policy of earlier ART initiation is neutral at worst—both in terms of cost and clinical outcomes—as it serves only to increase life expectancy among patients with less advanced disease.

Second, countries with currently one line of ART available but more resources should ensure access to CD4 count monitoring with ART initiation at CD4<350 cells/μl and then switch from stavudine to tenofovir, before making second-line ART available. Indeed, some countries have already responded to the 2010 WHO guidelines and have made plans to phase out stavudine [31]. Reductions in the price of tenofovir could resolve the ongoing debate surrounding the role for stavudine in resource-limited settings. At an annual cost of US$51, tenofovir would be both less costly and more effective than stavudine.

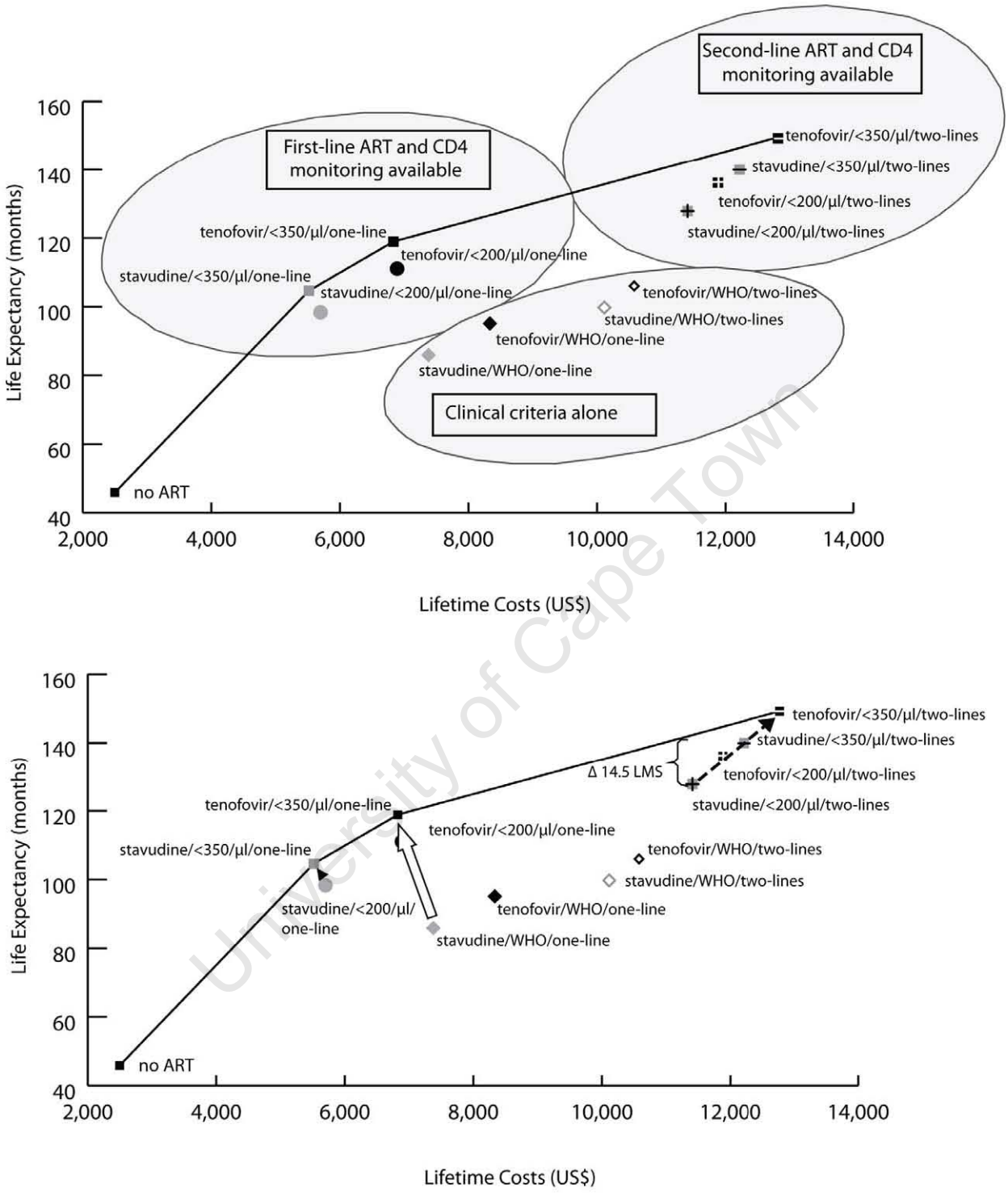

Figure 3. Clinical and economic outcomes of each of the scale-up interventions. The clinical and economic outcomes of all combinations of scale-up interventions are examined. The efficient frontier (marked by the line) connects the non-dominated strategies in the cost-effectiveness plane. Strategies below and to the right of the efficient frontier are those that are either strongly or weakly dominated by other options (see Methods). As illustrated in the upper panel, strategies based on clinical criteria (WHO stage III/IV) alone fall far below the efficient frontier (lower right oval), indicating their relatively high cost for the comparative benefit gained. Strategies in the upper left oval are those representing CD4 monitoring and one line of ART. Strategies incorporating a second-line regimen (upper right oval) all confer large survival benefits but at increased costs. The lower panel examines potential country situations. For instance, a country with a current stavudine/WHO/one-line policy could switch to a tenofovir/<350/µl/one-line policy (open arrow) and both decrease projected per-person lifetime costs and improve survival. A country with a stavudine/<200/

μl/one-line policy could decrease per-person costs and also improve outcomes by changing to a stavudine/<350/μl/one-line policy (solid arrowhead). Countries with a stavudine/<200/μl/two-lines policy would require increased per-person expenditures to achieve the survival benefits associated with tenofovir/<350/μl/two-lines (dotted arrow). To illustrate the impact of a policy requiring that all persons receive the same intervention, we examine the arbitrary affordability threshold of US$11,500 per person. The bracket (upper right) denotes the per person survival loss (14.5 mo) attributable to a policy requiring that all persons receive the same intervention.
doi:10.1371/journal.pmed.1000382.g003

Third, in countries with sufficient budgets to provide second-line ART, it is neither effective nor cost-effective to maintain stavudine in first-line regimens. Second-line ART may offer additional efficiencies by decreasing the prevalence of resistant virus and leaving future drug regimen options available.

Once countries have the capacity to provide early ART initiation, tenofovir, and second-line regimens, there will be additional clinical and policy questions. Policy makers will be addressing what to do upon second-line failure; optimal third-line regimens will be in question. Expanded ART regimen availability leads to clinical need for timely ART switches and forces the issue of HIV RNA laboratory availability. Finally, timely ART initiation is currently limited by late presentation to care [32,33]. Concurrent with scaling up to achieve the 2010 WHO ART guidelines, there should be a concerted effort to achieve the 2007 WHO HIV screening guidelines [34]; without earlier case detection, a policy of ART initiation at CD4<350 cells/μl will never be effectively realized.

It is important to highlight that full and immediate implementation of the comprehensive set of new guidelines is cost-effective by South African standards. But, while it is helpful to critically examine the survival and economic efficiency of alternative programmatic choices, "cost-effective" does not mean "affordable." In the setting of clear budget constraints, the question of affordability may conflict with the political imperative that all persons receive the same care package. In this case, prioritization of equity over efficiency decreases mean life expectancy—sometimes by more than 1 y per person—for the same healthcare expenditure (Figure 3, lower panel).

This analysis has several limitations. We report results from a cohort of HIV-infected individuals initiating ART. Although we believe the overall results would be consistent, this analysis does not specifically address ART programs with patients already in alternative stages of care, including some on first-line regimens, some on second-line regimens, and some who have previously accumulated drug-related toxicities. Such diversity within a cohort would require more individualized analyses. Additionally, a full budget impact analysis would be required to examine the number of patients in need of care, and to project the implications of each component of the WHO recommendation on program budgets over alternative time horizons.

Despite its limitations, this analysis represents the only systematic, scientific effort we are aware of that marshals the evidence base in support of implementing the WHO guidelines. The most unfortunate outcome upon release of the revised WHO guidelines would be either their complete dismissal on cost grounds alone, or the execution of more expensive—though easier to implement—interventions that offer less overall health benefit than other interventions.

In cases where the simultaneous implementation of all components of the 2010 WHO ART guidelines is beyond the reach of programs or countries, important prioritization questions emerge. This analysis suggests that CD4 count monitoring and ART initiation at CD4<350 cells/μl are the critical initial priorities. Replacing stavudine with tenofovir would further increase survival and would also be cost-effective. Adding a second-line ART regimen would provide large survival benefits, but with substantial increases in the necessary budgets.

Supporting Information

Figure S1 ART scale-up strategies.
Found at: doi:10.1371/journal.pmed.1000382.s001 (0.62 MB DOC)

Figure S2 Course of disease.
Found at: doi:10.1371/journal.pmed.1000382.s002 (0.22 MB TIF)

Figure S3 Validation of South African natural history data in the CEPAC model.
Found at: doi:10.1371/journal.pmed.1000382.s003 (0.33 MB TIF)

Figure S4 Patient survival in the first 5 y after model entry.
Found at: doi:10.1371/journal.pmed.1000382.s004 (0.41 MB TIF)

Text S1 WHO priorities: Technical appendix.
Found at: doi:10.1371/journal.pmed.1000382.s005 (0.27 MB DOC)

Text S2 WHO priorities; sensitivity analyses addendum, part 1.
Found at: doi:10.1371/journal.pmed.1000382.s006 (0.26 MB DOC)

Text S3 WHO priorities; sensitivity analyses addendum, part 2.
Found at: doi:10.1371/journal.pmed.1000382.s007 (0.12 MB XLS)

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Author Contributions

ICMJE criteria for authorship read and met: RPW RW ALC ADP SBL XA AWS KAF. Agree with the manuscript's results and conclusions: RPW RW ALC ADP SBL XA AWS KAF. Designed the experiments/the study: RPW RW. Analyzed the data: RPW ALC SBL KAF. Collected data/did experiments for the study: RPW XA. Wrote the first draft of the paper: RPW. Contributed to the writing of the paper: RW ALC ADP SBL XA KAF. Contributed to analysis and interpretation of results: ALC ADP KAF. Developed the model: AWS.

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Editors' Summary

Background. Since 1981, acquired immunodeficiency syndrome (AIDS) has killed more than 25 million people, and about 33 million people (30 million of them in low- and middle-income countries) are now infected with the human immunodeficiency virus (HIV), which causes AIDS. HIV destroys immune system cells (including CD4 cells, a type of lymphocyte), leaving infected individuals susceptible to other infections (so-called opportunistic infections). Early in the AIDS epidemic, most people with HIV died within 10 years of infection. Then, in 1996, highly active antiretroviral therapy (ART)—a combination of several powerful antiretroviral drugs—was developed. Now, in resource-rich countries, clinicians care for people with HIV by prescribing ART regimens tailored to each individual's needs. They also regularly measure the amount of virus in their patients' blood, test for antiretroviral-resistant viruses, and monitor the health of their patients' immune systems through regular CD4 cell counts. As a result, the life expectancy of patients with HIV in developed countries has dramatically improved.

Why Was This Study Done? Initially, resource-limited countries could not afford to provide ART for their populations, and the life expectancy of HIV-positive people remained low. Now, through the concerted efforts of governments, the World Health Organization (WHO), and other international agencies, more than a third of the people in low- and middle-income countries who need ART are receiving it. However, many without access are still in need of ART, and ART programs in developing countries follow a public-health approach rather than an individualized approach. That is, drug regimens, clinical decision-making, and disease monitoring are all standardized and follow recommendations in the 2006 WHO ART guidelines. This year (2010), these guidelines were revised. The guidelines now recommend the following: earlier ART initiation—when the CD4 count falls below 350/μl of blood, instead of below 200/μl as in the 2006 guidelines; the provision of sequential ART regimens instead of a single regimen; and the replacement of the antiretroviral drug stavudine with tenofovir, a less toxic but more expensive drug, in first-line ART regimens. However, many resource-limited countries are still struggling to implement the 2006 guidelines, so which of these new recommendations should be prioritized? Here, the researchers use a mathematical model to address this question.

What Did the Researchers Do and Find? The Cost Effectiveness of AIDS Complications (CEPAC)–International model simulates the natural history and treatment of HIV disease. The researchers entered South African clinical and cost data for HIV treatment into this model and then used it to project survival and costs in a hypothetical group of South African HIV-positive patients under alternative guideline prioritization scenarios. The reference strategy for the simulations (denoted as "stavudine/WHO/one-line") assumed that patients (with a mean CD4 count of 375/μl) began a single stavudine-based ART regimen when they developed WHO stage III/IV HIV disease (i.e., when patients develop multiple opportunistic infections such as tuberculosis and pneumonia). When the new guideline recommendations were considered separately, ART initiation at CD4<350/μl (stavudine/<350/μl/one-line) maximized five-year survival. Stepwise adjustment from the reference strategy (which had a life expectancy 99.0 months) through strategies of stavudine/<350/μl/one-line (a projected life expectancy of 124.3 months), stavudine/<350/μl/two-lines (177.6 months), and tenofovir/<350/μl/two-lines (193.6 months) produced the greatest improvements in life expectancy. Finally, strategies of stavudine/<350/μl/one-line, tenofovir/<350/μl/one-line, and tenofovir/<350μl/two-lines produced incremental cost-effectiveness ratios of US$610, US$1,140, and US$2,370 per year of life saved, respectively.

What Do These Findings Mean? As with all mathematical models, the accuracy of these findings are dependent on the assumptions included in the model and on the data populating it. Nevertheless, these findings suggest that, where resources are limited and immediate implementation of all the new WHO recommendations is impossible, ART initiation at a CD4 count of less than 350/μl would provide the greatest survival advantage and would be very cost-effective. In countries that are already initiating ART at this threshold and that have access to CD4 monitoring, a switch from stavudine to tenofovir would further increase survival and would also be cost-effective. Finally, although access to a second-line ART regimen would provide more clinical benefits than access to tenofovir, the cost of this change in strategy would be substantially greater. Importantly, these findings should help policy makers adjust their ART program strategies to maximize their clinical benefits and cost effectiveness.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1000382.

- Information is available from the US National Institute of Allergy and Infectious Diseases on HIV infection and AIDS
- HIV InSite has comprehensive information on all aspects of HIV/AIDS
- Information is available from Avert, an international AIDS charity, on many aspects of HIV/AIDS, including information on HIV/AIDS in South Africa and on HIV/AIDS treatment and care (in English and Spanish)
- WHO provides information about universal access to AIDS treatment (in English, French, and Spanish); its 2010 ART guidelines can be downloaded
- More information on the CEPAC model is available

Early mortality among adults accessing a community-based antiretroviral service in South Africa: implications for programme design

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Objectives: To determine rates, risk factors and causes of death among patients accessing a community-based antiretroviral treatment (ART) programme both prior to and following initiation of treatment.

Methods: All in-programme deaths were ascertained between September 2002 and March 2005 among treatment-naive patients enrolled into a prospective community-based ART cohort in Cape Town, South Africa.

Results: Of 712 patients (median CD4 cell count, 94 cells/μl), 578 (81%) started triple ART a median of 29 days after enrolment. 68 (9.5%) patients died during 563 person-years of observation. The high pretreatment mortality rate of 35.6 deaths/100 person-years [95% confidence interval (CI), 23.0–55.1) decreased to 2.5/100 person-years (95% CI, 0.9–6.6) at 1 year among those who received ART. However, within the first 90 days from enrolment, 29 of 44 (66%) deaths occurred among patients awaiting ART; these would not be identified by an on-treatment analysis. Multivariate analysis showed that risk of death (both pre-treatment and on-treatment) was independently associated with baseline CD4 cell count and World Health Organization (WHO) clinical stage; stage 4 disease was the strongest risk factor. Major attributed causes of death were wasting syndrome, tuberculosis, acute bacterial infections, malignancy and immune reconstitution disease.

Conclusions: Most early in-programme deaths occurred among patients with advanced immunodeficiency but who had not yet started ART. Programme evaluation using on-treatment analyses greatly underestimated early mortality. This mortality would be reduced by minimizing unnecessary in-programme delays in treatment initiation and by starting ART before development of WHO stage 4 disease.

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Introduction

While there is ample evidence that use of antiretroviral treatment (ART) dramatically improves the prognosis of individuals with HIV infection [1–4], the vast majority of those requiring ART in sub-Saharan Africa and other resource-limited settings do not have access to this treatment. To address these inequities in treatment availability, a number of initiatives have been developed to expand ART access [5]. The collective goal of these initiatives under the umbrella of the World Health Organization (WHO) is to provide ART to 3 million

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people by the end of 2005 [5]. While progress towards this goal is being achieved, it was estimated that just 310 000 (< 8%) of the 4 million patients aged 15–49 years in sub-Saharan Africa who needed treatment were receiving it by the end of 2004 [6].

The huge number of patients requiring treatment presents a formidable logistical challenge. Despite increasing allocation of resources to expand access to ART in resource-limited settings, little is known about how best to deliver treatment services. It is becoming increasingly clear that the impact of ART services at a population level will primarily be determined not by issues of drug efficacy [7] but rather by programmatic issues of treatment availability, accessibility and delivery [8]. Data from existing ART programmes are urgently required to identify approaches to optimize outcomes. Previous studies indicate that the first-year mortality from the time of initiation of ART is broadly similar among cohorts receiving ART in low- and high-income countries [9–14]. The current study examines not only mortality during treatment but also that occurring in the interval between eligible individuals being enrolled into the ART programme and the actual initiation of ART. Identification of risk factors and stratification of mortality data according to enrolment characteristics has also permitted evaluation of treatment criteria for patients in low-resource settings.

Methods

Antiretroviral treatment programme

The ART service described here is based at the Gugulethu Community Health Centre in Nyanga district, Cape Town [15]. This peri-urban district is home to a predominantly African population of over 300 000, the vast majority of whom live in conditions of low socioeconomic status. In 2003, the antenatal HIV seroprevalence was 28%. Ten primary care HIV clinics form the patient referral base and enrolment into the ART programme follows the Department of Health's national guidelines [16], which are based on the WHO recommendations (2002) [17]. These criteria include those with a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count < 200 cells/μl.

Following referral to the ART service, the standard schedule of visits was as follows: screening visit (week 0), blood tests for plasma HIV load and blood CD4 lymphocyte count (week 2), treatment initiation (week 4) and treatment follow up (weeks 8, 12 and 20, and 16-weekly thereafter). At the screening visit, a treatment readiness evaluation was completed and a 4-week supply of co-trimoxazole was dispensed, with pill counts at 14 and 28 days to assess adherence. Patients were assessed by a doctor for symptomatic HIV-associated disease.

At the screening visit, patients were also allocated a therapeutic counsellor living in the same community whose role was to use clinic and home visits to provide ongoing counselling and adherence support. These counsellors provided weekly updates to the multidisciplinary team about the status of patients, including information regarding those who failed to attend follow up appointments [16]. The treatment readiness status of patients was reviewed each week until either treatment was started or the patient was permanently deferred or had died. Potential reasons for temporary deferral of treatment included current investigation of an intercurrent opportunistic infection, lack of patient readiness or a failure to attend follow-up appointments. Reasons for permanent deferral of patients either before or after commencing treatment included decision to access treatment elsewhere, recurrent failure to attend follow up clinic appointments, relocation out of area and psychosocial reasons such as denial of HIV infection status.

First-line ART comprised stavudine, lamivudine plus a non-nucleoside reverse transcriptase inhibitor (efavirenz or nevirapine). The second-line regimen for those failing the first-line treatment comprised lopinavir/ritonavir, zidovudine and didanosine. Treatment adherence and viral load suppression < 400 copies/ml in this cohort both exceed 90% at 1 year [18]. All treatment was free of charge and there were no interruptions in drug supply. All patients with CD4 cell counts < 200 cells/μl received daily co-trimoxazole prophylaxis; dapsone was used as an alternative. In addition to the scheduled clinic appointments, patients had open access to the clinic for medical problems. Patients requiring in-patient care were referred to a 200-bed secondary hospital nearby.

Data sources

Structured clinical records were maintained on all patients screened on entry to the ART programme and this information was transferred on a weekly basis to a database. Where blood CD4 cell counts and viral load measurements from the week 2 visit were missing, the results of tests done at the local referring centre within the 3 months prior to the screening visit were used where available.

Information regarding deaths of patients within the care of the ART clinic was obtained from the local secondary and tertiary care hospitals, hospital mortality review meetings and postmortem examinations. The most likely attributable cause of death of each patient was assigned based on all the available information after detailed review by two specialists in infectious diseases and HIV medicine.

Data analysis

Data were analysed using STATA version 8 (College Station, Texas, USA). Wilcoxon rank sum and Fisher's exact tests were used to compare medians and proportions, respectively. For the main analysis, person-time

was calculated from the date of initial screening by the service until the earliest of the following dates: (a) permanent deferral from the ART service; (b) death, or (c) date of data censorship (March 2005) for those alive and still enrolled into the programme. Stratified analyses were used to compare mortality rates by 90-day intervals after enrolment into the programme, WHO stage, baseline CD4 cell count, and for person-time while receiving and or not receiving ART. Additional analyses were restricted to individuals receiving ART, using person-time calculated from the date of ART initiation until death, permanent deferral or censorship of the dataset. Kaplan–Meier analyses with log rank tests were used to examine the effect of patient characteristics on survival probabilities. All rates are reported per 100 person-years and all statistical tests were two sided at $\alpha = 0.05$.

Multivariate analyses modelled the association between mortality rate, treatment status (based on person-time in the programme while receiving or not receiving ART), WHO clinical stage and CD4 cell count at screening, and the age and gender of the participants. To account for intraindividual correlations (on and off treatment) a population-average log-linear model was used with an exchangeable working correlation structure and sandwich (robust) estimators. The results are presented as mortality rate ratios with corresponding 95% confidence intervals (CI).

Results

Enrolment and follow up

Between September 2002 and February 2005, 758 individuals were referred for ART. Of these, the following were excluded from the study analysis: patients < 18 years of age ($n = 16$); those transferred into the ART service having previously initiated treatment elsewhere ($n = 6$) and those ineligible for ART according to programme criteria ($n = 24$). The remaining 712 subjects were included in this analysis. Of these, 527 (74%) were female and the median age was 33 years [interquartile range (IQR), 28.5–38]. Baseline blood CD4 lymphocyte counts and plasma viral load measurements were available for 675 (95%) and 647 (91%) of subjects, respectively. The median blood CD4 lymphocyte count was 94 cells/μl; the numbers of patients with CD4 cell counts < 50, 51–100, 101–150 and > 151 cells/μl were 187 (28%), 163 (24%), 152 (23%) and 173 (26%), respectively. The median plasma viral load was 72 349 copies/ml (IQR, 30 721–191 000). Disease was categorized as WHO clinical stage 1 for 63 subjects (9%), stage 2 for 78 (11%), stage 3 for 354 (50%) and stage 4 for 215 (30%); data were not recorded for two subjects (0.3%).

After screening, 578 patients (81%) started ART. The median interval between screening and initiation of treatment was 29 days: 75% of patients started treatment within 6 weeks (42 days) and 96% within 3 months (90 days). The length of this interval was not associated with blood CD4 cell count or viral load. A total of 563 person-years of observation accrued during follow-up, of which 488 person-years were during ART, and the median period of observation during treatment was 284 days (IQR, 124–510) with a maximum of 925 days (2.5 years). Twenty-two treated patients (4%) were lost to follow up through transfer to another programme ($n = 3$) or failure to attend follow-up appointments ($n = 19$; 3%).

Among 134 patients (19%) who did not receive ART, the most frequent reasons for this were death, decision to access treatment elsewhere, failure to attend follow up clinic appointments, moving out of the area and psychosocial reasons such as denial of HIV infection status. The median period of observation for these patients was 28 days (IQR, 19–50; maximum, 125 days). The total untreated patient time was 75 person-years and was made up of that for patients who did not receive ART as well as that for patients who subsequently received ART.

Death rates

Sixty eight (9.5%) patients died following enrolment into the programme, with an all-cause mortality rate of 12.1 deaths/100 person-years (95% CI, 9.5–15.3) (Table 1). The baseline pretreatment mortality rate (during the first 30 days of entry to the programme) was very high (35.6 deaths/100 person-years; 95% CI, 23.0–55.1) but the overall rate decreased markedly during follow up (Table 1). Forty-four (65%) of the deaths occurred within the first 90 days from enrolment. Among those who received ART, the mortality rate during the first month of treatment (17.5 deaths/100 person-years, 95% CI, 8.8–35.0) was 2.03-fold (95% CI, 0.93–5.79) lower than the baseline rate. The mortality rate continued to decrease during ART, and after 6–9 months the rate was 13.2-fold lower than the baseline rate. The survival probability among treated patients at 1 year was 0.929 (Fig. 1). Deaths among patients who did not start ART was very high (Fig. 2; Table 1) and 31 patients (5.4% of enrolled patients) died before they were able to start ART. Most importantly, among the 44 patients who died within 3 months of enrolment, 29 (66%) were not receiving ART and would have been excluded from data evaluating the programme by an on-treatment analysis.

Risk factors for mortality

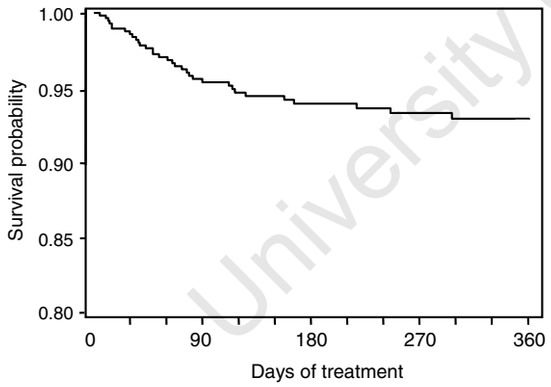
It was hypothesized that the very high early mortality rate was primarily related to enrolment of patients with very advanced immunodeficiency; therefore, the relationship was examined between survival probability and baseline WHO clinical stage of disease or the blood CD4 cell count. Among those who received ART, no deaths

Table 1. Distribution of deaths by 90–day intervals.

	Among all patients (from screening)			Person-time on ART (from start of treatment)			Person-time not on ART (from screening)		
Interval (days)	Deaths	Person-days	Rate (95% CI)[a]	Deaths	Person-days	Rate (95% CI)[a]	Deaths	Person-days	Rate (95% CI)[a]
1–90	44	53 919	29.8 (22.2–40.0)	24	45223	19.4 (13.0–28.9)	29	26122	40.5 (28.2–58.3)
91–180	11	38 555	10.4 (5.8–18.8)	6	35110	6.2 (2.8–13.9)	2	777	94.0 (23.5–375.7)
181–270	7	30 699	8.3 (4.0–17.5)	2	27472	2.7 (0.7–10.6)	–	28	–
271–360	2	23 672	3.1 (0.8–12.3)	1	20486	1.8 (0.3–12.7)	–	–	–
> 360	4	58 612	2.5 (0.9–6.6)	4	49873	2.9 (1.1–7.8)	–	–	–
Total	68	205 457	12.1 (9.5–15.3)	37	178164	7.6 (5.5–10.5)	31	26927	41.5 (29.2–59.8)

ART, antiretroviral therapy; CI, confidence interval.
[a]All-cause mortality rate per 100 person-years.

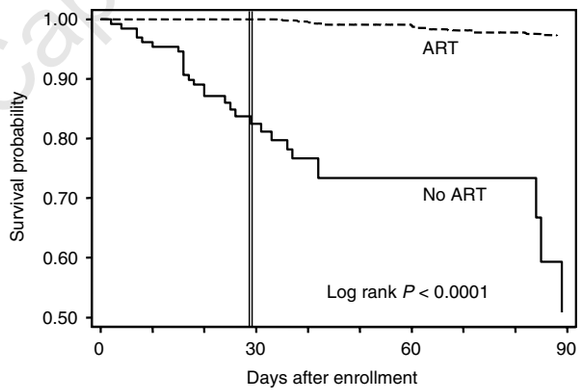
occurred among those with stage 1 or 2 disease; in contrast, patients with stage 3 and stage 4 disease had an incrementally greater risk of death (Fig. 3a). Increasing risk of death was also significantly associated with decreasing baseline CD4 cell count (Fig. 3b). Similar significant associations were seen among those who did not receive ART (data not shown). Among all patients enrolled into the programme, the median blood CD4 cell count of those who died was lower than that of those who survived (54 versus 98 cells/μl; $P < 0.001$). Furthermore, patients who died were more likely to have stage 4 disease than those who did not die (37/68 [54%] versus 178/644 [28%]; $P < 0.0001$).



Start of interval	0 days	90 days	180 days	270 days	> 360 days
Eligible at start of interval	578	483	350	269	191
Deaths during interval	24	6	2	1	4
Survival probability at start of interval	1.0	0.953	0.939	0.932	0.929

Fig. 1. Kaplan–Meier curve for patients who received antiretroviral treatment showing probability of survival from initiation of treatment.

Kaplan–Meier survival curves for all patients enrolled into the programme show that stage 4 disease and baseline blood CD4 cell count ≤ 50 cells/μl were both associated with a substantially lower survival probability compared with other patients (Fig. 3c,d). Among the 44 deaths that occurred within the first 3 months of the programme, 35 (66%) had stage 4 disease and 22 (50%) had blood CD4 cell counts < 50 cells/μl. Overall, patients with stage 4



Start of interval	0 days	30 days	60 days	> 90 days
No ART				
Eligible at start of interval	134	62	23	7
No. of deaths	20	6	3	2
Survival probability at start of interval	1.0	0.82	0.73	0.51
Receiving ART				
Eligible at start of interval	578	556	479	432
No. of deaths	0	7	8	22
Survival probability at start of interval	1.0	1.0	0.99	0.97

Fig. 2. The effect of receiving antiretroviral therapy. Kaplan–Meier curves for patients who did (ART) or did not (No ART) receive treatment, showing the probability of survival from entry to the programme. The median interval between enrolment and treatment initiation is indicated by a double vertical line at 29 days.

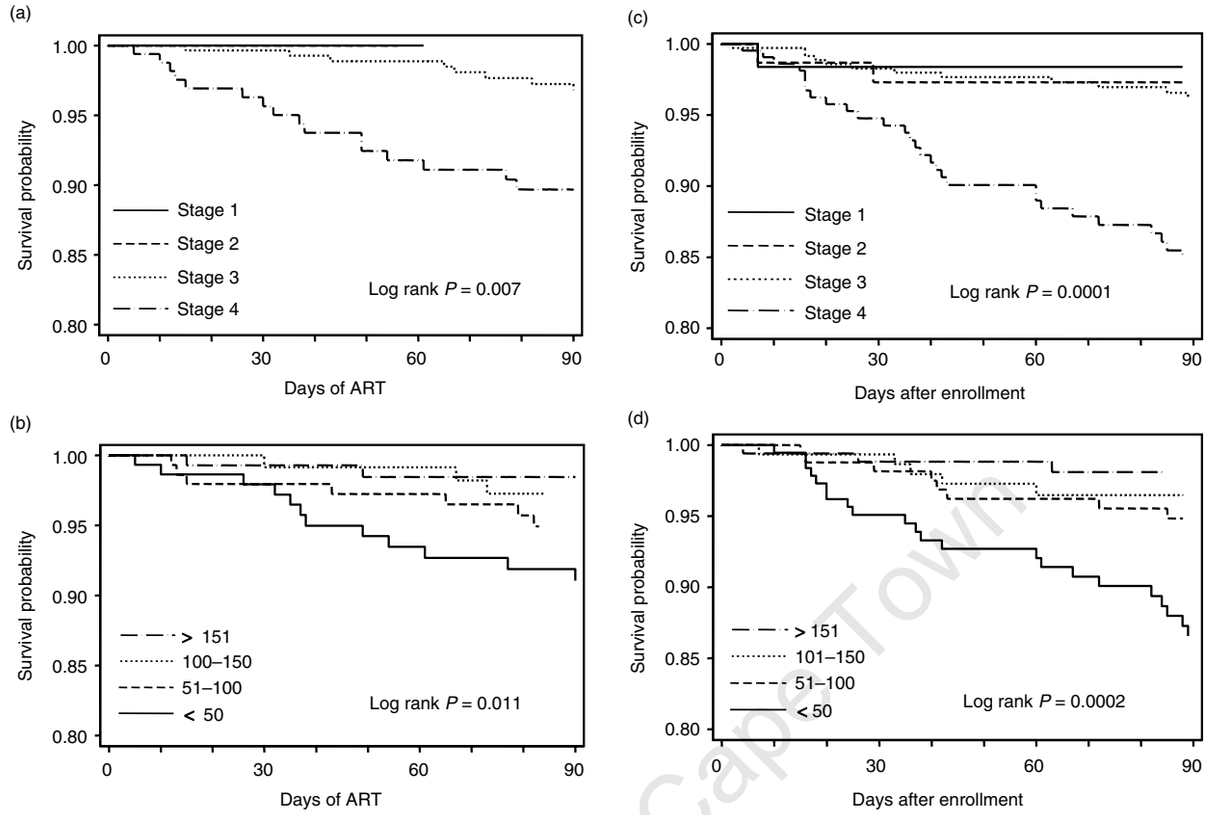


Fig. 3. The effect of disease stage on survival in the programme. (a,b) Survival in patients who received antiretroviral treatment; Kaplan–Meier plots showing the probability of survival from initiation of treatment stratified by baseline World Health Organization stage (a) and blood CD4 lymphocyte count (b). (c,d) Survival for all patients enrolled into the programme; Kaplan–Meier plots showing the probability of survival from enrolment stratified by baseline WHO stage (c) and blood CD4 cell count (d). CD4 lymphocyte count groups were stratified as > 151, 101–150, 51–100 and < 50 cells/μl.

disease and/or blood CD4 cell counts < 50 cells/μl accounted for 80% of the deaths within 3 months of programme entry. In contrast, patients with CD4 cells counts > 150 cells/μl had a low risk of death (Fig. 3d). Having adjusted for the effects of treatment, multivariate analysis showed that the mortality rate was not independently associated with age or sex but was associated with disease stage and blood CD4 cell count. Compared with patients with stages 1 and 2 disease, those with stage 3 disease and stage 4 disease had mortality rate ratios of 3.44 (95% CI, 0.80–14.85) and 5.93-fold (95% CI, 1.36–25.89), respectively. Similarly, the mortality rate ratio comparing those with CD4 cell counts < 50 and > 50 cells/μl was 3.34 (95% CI, 1.31–8.50).

Cause of death

Likely causes were identified for 61 of the 68 (89.7%) deaths. Postmortem examinations were carried out for six patients. Almost two-thirds of deaths were attributable to wasting syndrome, tuberculosis, acute bacterial infections and malignancy (Table 2). ART was associated with a smaller proportion of deaths from acute respiratory infections even though antibiotic prophylaxis was received by all patients both before and during ART. Of particular note, six cases of microbiologically proven cryptococcal meningitis occurred, all among patients within the initial weeks of ART. Of these, four were receiving secondary prophylaxis with fluconazole. The temporal association between initiation of ART and development of fulminant cryptococcal meningitis strongly implicated immune reconstitution disease as a cause for this presentation. Three other deaths among patients receiving ART were also thought to be related to immune reconstitution disease, including two associated with tuberculosis and one with Kaposi's sarcoma. Of these, two were diagnosed by postmortem examination. Overall, immune reconstitution disease was thought to have contributed to 9 of the 37 (24%) deaths during ART. Among three drug-related deaths (Table 2), two were among patients receiving ART: one patient developed nevirapine-induced skin rash complicated by septicaemia and another receiving nucleoside analogue reverse transcriptase inhibitors developed laboratory-confirmed lactic acidosis.

Table 2. Causes of in-programme deaths.

Cause of death	Deaths [No. (%)]		
	All patients ($n = 68$)	Patients receiving ART ($n = 37$)	Patients not receiving ART ($n = 31$)
Wasting syndrome	13 (19.1)	5 (13.5)*	8 (25.8)*
Acute infection			
Acute respiratory infection	7 (10.3)	1 (2.7)**	6 (19.4)**
Acute gastroenteritis	4 (5.9)	2 (5.4)	2 (6.5)
Systemic sepsis	2 (2.9)	2 (5.4)	0 (0)
Tuberculosis	10 (14.7)	5 (13.5)	5 (16.1)
Malignancy			
Kaposi's sarcoma	7 (10.3)	4 (10.8)	3 (9.7)
Lymphoma	1 (1.5)	1 (2.7)	0 (0)
Cryptococcal meningitis	6 (8.8)	6 (16.2)***	0 (0)[c]
Chronic respiratory disease (non TB)	5 (7.4)	4 (10.8)	1 (2.3)
Drug adverse effects	3 (4.4)	2 (5.4)	1 (3.2)
Miscellaneous	4 (5.9)	3 (8.1)	1 (3.2)
Unknown	6 (8.8)	2 (5.4)	4 (12.9)

*$P = 0.230$;
**$P = 0.041$;
***$P = 0.037$ (Fisher's exact test).

Discussion

This study defined mortality rates among patients accessing a community-based public sector ART programme in South Africa and who were eligible for treatment under the WHO 2002 guidelines [17]. This is the first study in a resource-limited setting to report not only mortality rates among patients during ART but also rates in the interval between enrolment into the programme and the actual start of treatment. Our data highlight substantial 'unseen' in-programme mortality that occurs within the interval between enrolment into the programme and initiation of ART, providing important additional insights beyond those provided by previous studies reported from sub-Saharan Africa [9–14]. Furthermore, we identified causes of death among the majority of patients and were able to determine the mortality risk associated with differing levels of baseline immunodeficiency, thereby providing an evaluation of ART enrolment criteria. These analyses were made possible by rigorous prospective data collection. Use of community-based therapeutic counsellors allocated to every patient greatly enhanced the data completeness and assignment of outcomes for patients who failed to attend follow up appointments. Since triple-drug ART and prophylactic co-trimoxazole were provided free of charge to all patients, data were not limited by a variable standard of care based upon the financial resources of the patients.

The baseline death rate among patients enrolled into the programme was extremely high. Following development of AIDS, the median survival of untreated patients in South Africa and rural Uganda is just 9–10 months [19,20] compared with around 2 years in high-income countries prior to the advent of ART [21–23]. A collaborative analysis of datasets from cohorts receiving ART in northern and southern hemisphere countries also shows that early on-treatment mortality rates among patients with advanced baseline immunodeficiency were much higher in low-income countries than in high-income countries despite similar virological and immunological responses to treatment [24].

A substantial reduction in mortality associated with ART was evident within the first months of treatment and the probability of survival on treatment at 1 year was high (0.929). However, our most important finding was that 66% of the patients who died within 90 days of enrolment to the programme were not yet receiving ART. The interval from enrolment to starting treatment in this study (median, 29 days; 75th centile, 42) was short compared with the minimum of 4 months lead-in time at a similar community-based antiretroviral programme in South Africa [9] and is likely to compare favourably with many programmes in the region. The pretreatment interval in this programme permitted clinical assessment, blood testing, patient attendance at a structured education programme, arrangement of a home assessment by the therapeutic counsellor, assessment of treatment compliance using co-trimoxazole pill counts and procurement of drug. Thorough preparation of patients for treatment in this way has been associated with very high treatment compliance rates and excellent virological response rates [18,25].

An important implication of this study is that careful consideration needs to be given to the design of community-based ART programmes for low-income countries. Although it cannot be concluded from the data here, minimization of the in-programme pretreatment interval may well decrease mortality in this and other programmes. However, a balance needs to be established between minimizing the pretreatment interval (potentially reducing early mortality risk) and allowing adequate

time to prepare patients for treatment (promoting high rates of treatment adherence and reducing long-term mortality rates). A system might also be employed whereby patients at highest risk of death are 'fast-tracked' onto treatment; such patients might include those with stage 4 disease, a blood CD4 lymphocyte count < 50 cells/μl or an AIDS-defining illness associated with a particularly poor prognosis, such as wasting syndrome [26,27]. Data from other ART services in resource-limited settings may help to identify optimal strategies. A further implication of these data is that failure to record in-programme pretreatment deaths may have resulted in survival bias in previous reports, leading to an over-estimation of survival benefits among those with advanced immunodeficiency. Overestimation of the benefits of ART among such patients may unwittingly tend to skew priorities away from treating patients at an earlier stage of disease.

The extremely high mortality in the first 30 days from enrolment suggests that many patients enrolling into the programme had disease that was too far advanced at entry. This is clearly supported by the finding that risk of death was very strongly associated with baseline blood CD4 cell count and WHO clinical stage of disease. Indeed, 80% of deaths within the first 3 months from enrolment were among those with stage 4 disease or a baseline blood CD4 cell count of < 50 cells/μl. The South African ART roll-out programme currently employs the WHO 2002 guidelines, recommending treatment for those with a clinical criterion of stage 4 disease or a laboratory criterion of a CD4 cell count < 200 cells/μl. We found that in-programme mortality among those with CD4 cell counts > 151 cells/μl was low (Fig. 3d), suggesting that initiation of ART among patients with CD4 cells counts of 150–200 cells/μl would be an acceptable target range for initiating treatment based on mortality outcomes. In contrast, however, the in-programme mortality rate among those with stage 4 disease was unacceptably high (Fig. 3c). Once patients developed stage 4 disease, the mortality rate is already so high that the inevitable delays inherent in accessing care, diagnosis, referral to an ART programme, preparation for treatment and actually initiating ART result in an unacceptable mortality rate, of the order of 7% per month [19]. Based on these data, the clinical criterion for initiating ART should be when patients first develop symptomatic (stage 3) disease.

Tuberculosis is the most frequent manifestation of WHO stage 3 disease in the region and the tuberculosis treatment programmes could provide a key point of access to the ART programme. Therefore, recommending treatment for all patients with symptomatic (stage 3 and stage 4) disease and those whose CD4 cell counts < 200 cells/μl may reduce mortality. In the face of resource limitations, such a policy would avoid the even greater expansion of patient numbers requiring treatment that would result from recommendations to treat patients with CD4 cell counts < 350 cells/μl as is practice in high-income countries.

Cause-specific mortality data for HIV-infected individuals living in sub-Saharan Africa are lacking. Postmortem studies of selected in-patient deaths in Côte D'Ivoire and Botswana found that the predominant causes were tuberculosis, bacterial pneumonia and cerebral toxoplasmosis [28,29]. Active tuberculosis was also detected by postmortem examinations among nearly one half of patients dying with HIV wasting syndrome in West Africa [30]. In our cohort, in which prophylaxis with co-trimoxazole (or dapsone) was universally prescribed, 68% of deaths among patients not receiving HAART were associated with wasting syndrome, tuberculosis and acute bacterial infections. The most striking difference in mortality pattern, comparing those who did and did not receive ART, was that the six deaths from cryptococcal meningitis all occurred among patients within the first few weeks of ART, strongly suggesting immune reconstitution disease as a possible cause [31]. Overall immune reconstitution disease was thought likely to have contributed to over 20% of deaths within the first 3 months of ART. This is typically a complication of patients with advanced immunosuppression at the time ART is started and this phenomenon provides another argument favouring treatment of patients earlier in the course of disease.

In summary, this analysis found that most early in-programme deaths occurred among patients with advanced immunodeficiency who had not yet initiated ART. If this observation is shared by other programmes, then previously published programme evaluations using on-treatment analyses may have greatly underestimated early in-programme mortality. Unnecessary in-programme delays in treatment initiation should be minimized. In addition, we suggest that that the programmatic application of the WHO 2002 treatment guidelines is associated with an unacceptably high mortality rate in this setting. Strategies to reduce early mortality should include treatment of all patients with symptomatic (stage 3 and stage 4) disease.

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Mortality of HIV-1 patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries

The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration and ART Cohort Collaboration (ART-CC) groups

Summary

Background **Highly active antiretroviral therapy (HAART) is being scaled up in developing countries. We compared baseline characteristics and outcomes during the first year of HAART between HIV-1 patients in low-income and high-income settings.**

Methods **18 HAART programmes in Africa, Asia, and South America (low-income settings) and 12 HIV cohort studies from Europe and North America (high-income settings) provided data on 4810 and 22 217, respectively, treatment-naïve adult patients starting HAART. All patients from high-income settings and 2725 (57%) patients from low-income settings were actively followed-up and included in survival analyses.**

Findings **Compared with high-income countries, patients starting HAART in low-income settings had lower CD4 cell counts (median 108 cells/μL *vs* 234 cells/μL), were more likely to be female (51% *vs* 25%), and more likely to start treatment with a non-nucleoside reverse transcriptase inhibitor (NNRTI) (70% *vs* 23%). At 6 months, the median number of CD4 cells gained (106 cells/μL *vs* 103 cells/μL) and the percentage of patients reaching HIV-1 RNA levels lower than 500 copies/mL (76% *vs* 77%) were similar. Mortality was greater in low-income settings (124 deaths during 2236 person-years of follow-up) than in high-income settings (414 deaths during 20 532 person-years). The adjusted hazard ratio (HR) of mortality comparing low-income with high-income settings fell from 4.3 (95% CI** [A: ?ok] **1.6–11.8) during the first month to 1.5 (0.7–3.0) during months 7–12. The provision of treatment free of charge in low-income settings was associated with lower mortality (adjusted HR 0.23; 95% CI 0.08–0.61).**

Interpretation **Patients starting HAART in resource-poor settings have increased mortality rates in the first months on therapy, compared with those in developed countries. Timely diagnosis and assessment of treatment eligibility, coupled with free provision of HAART, might reduce this excess mortality.**

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Introduction

The increasingly widespread use of highly active antiretroviral therapy (HAART) since 1996 has substantially improved the prognosis of HIV-infected patients who have access to these drugs.[1-3] In resource-poor settings in Africa, Asia, and South America, where 90% of people with HIV/AIDS live, access to HAART is limited. With falling prices of proprietary drugs, the increasing availability of generic formulations and the launch of initiatives by international agencies, including the World Health Organization's (WHO's) "3 by 5" programme (to get 3 million HIV patients on antiretrovirals by 2005), the Global Fund to fight AIDS, Tuberculosis and Malaria, and the US President's Emergency Plan for AIDS Relief (PEPFAR), this situation is changing. The WHO estimates that as of June, 2005, about 1 million people were receiving HAART, although this number still only represents 15% of the estimated 6.5 million people in urgent need of antiretroviral therapy in low-income and middle-income countries.[4]

Several factors could limit the effectiveness of HAART in resource-poor settings. Interruptions in supply at the programme level or patients' limited financial resources might compromise adherence and treatment efficacy. The high prevalence of co-infections, notably with tuberculosis and other bacterial diseases might also affect prognosis.[5-7] Here we report on the Antiretroviral Therapy in Lower Income Countries (ART-LINC) collaboration, a network of treatment programmes in Africa, Asia, and South America.[8] Our objective was to compare early mortality and immunological and virological response in patients starting HAART in these settings with outcomes in patients participating in a similar collaboration of cohort studies in high-income countries, the ART Cohort Collaboration (ART-CC).[1]

Methods

Participants

Treatment programmes in low-income countries were identified by searching published scientific reports, including abstracts from recent conferences, and by consulting with colleagues. Site assessments were done with a standardised questionnaire. Programmes that collected prospective data on patient characteristics and outcomes were eligible for inclusion in ART-LINC. 23 treatment programmes were approached, 19 agreed to participate, and 18 of these **[A:?correct]** contributed data to this analysis.

Information obtained for patients included sociodemographic data, date of starting HAART, and,

where available, CD4 cell counts and HIV-1 RNA levels at baseline and during follow-up. Type of regimen was defined as protease inhibitor-based (one protease inhibitor and two nucleoside reverse transcriptase inhibitors [NRTIs], including ritonavir-boosted regimens), non-nucleoside reverse transcriptase inhibitors [NNRTI] based (one NNRTI and two NRTIs), or other combinations (including triple NRTI regimens and any other regimen containing a minimum of three drugs). Programme characteristics were also recorded, including the use of generic drugs, virological monitoring, costs to patients, tracing of patients who had been lost to follow-up, and other services, including voluntary counselling and testing, and tuberculosis clinics. We defined routine monitoring of virological response as at least one viral load measurement between 3 and 9 months after starting HAART, in at least 50% of patients.

The ART-CC is a collaboration of cohort studies and clinical databases from North America and Europe, established in 2001 to estimate prognosis in treatment-naïve HIV-infected patients initiating HAART. Eligibility criteria and methods have been reported elsewhere.[1,9] We included all patients in each of the two collaborations who had not previously received antiretroviral therapy, were aged 16 years or older, with known date of starting HAART and a documented baseline CD4 count. The stage of disease was classified as either less advanced (CDC stage A/B, WHO stage I/II) or more advanced (CDC stage C, WHO stage III/IV). The selection of patients and extraction of data was done at the participating centres. Anonymous data were pooled and analysed centrally. At all sites, institutional review boards had approved the collection of data. We use the terms low-income and high-income settings for the treatment programmes participating in the ART-LINC and ART-CC collaborations, respectively.

[A: For HIV-NAT, please provide the exact percentage. Please add in numbers where I have indicated XX]
[A: Pls spell out programme names in the legend]

Outcomes

The primary endpoint was mortality from all causes in the first year after starting HAART. Changes in CD4 cell counts in the first 6 months, and the proportion of patients with viral load less than 500 copies/mL at 6 months were secondary endpoints. Measurements closest to 6 months after starting HAART, within 3 to 9 months, were used in these analyses. We used an intent-to-continue treatment approach, and ignored changes to treatment, treatment interruptions and terminations. Time was measured from the start of HAART and ended at the earliest of: the date of death; the date of the last follow-up visit; or month 12 after starting HAART. A patient was judged to be lost to follow-up if the last visit was recorded during the first year after starting HAART and the patient had at least 1 year of additional potential follow-up until the closing date of the database. The closing date was defined for each cohort as the date of the most recent follow-up recorded in the database.

Statistical analysis

In ART-LINC, disease stage at the time of starting treatment was not available for all patients. Stage of disease is strongly associated with other variables—in particular, CD4 cell count. We therefore created multiple datasets in which disease stage was entered on the basis

	Country	Free access to treatment	Use of generic drugs	Routine virological testing	Voluntary counselling and testing	Tuberculosis clinic on site	n (%)	Active follow-up	Proportion lost to follow-up
Programme									
North Africa									
Morocco ART cohort	Morocco	Yes	No	Yes	No	Yes	300 (6%)	Yes	xx (4%)
Southern Africa									
Gaborone Independent	Botswana	No	No	Yes	Yes	No	209 (4%)	Yes	Xx (6%)
Lighthouse	Malawi	No	Yes	No	Yes	No	1056 (22%)	No	XX (31%)
CTAC	South Africa	Yes	No	Yes	Yes	No	305 (6%)	Yes	Xx (15%)
Khayelitsha	South Africa	Yes	Yes	Yes	Yes	Yes	278 (6%)	Yes	0%
OPERA	South Africa	Yes	No	Yes	Yes	No	53 (1%)	Yes	0%
East Africa									
Nsambya	Uganda	No	Yes	No	Yes	Yes	236 (5%)	Yes	XX (44%)
GATP Kampala	Uganda	No	Yes	Yes	No	No	74 (2%)	Yes	0%
Eldoret	Kenya	Yes	Yes	No	Yes	Yes	663 (14%)	Yes	XX (14%)
Central and west Africa									
Parvy	Cameroon	Yes	Yes	Yes	Yes	Yes	115 (2%)	Yes	XX (44%)
COTRAME	Côte d'Ivoire	Yes	No	No	Yes	Yes	131 (3%)	Yes	0%
Nigeria HAART	Nigeria	No	Yes	No	Yes	Yes	102 (2%)	Yes	0%
ISAARV	Senegal	Yes	Yes	Yes	Yes	Yes	146 (3%)	Yes	XX (11%)
HIMS	Various	Yes	Yes	No	Yes	No	81 (2%)	Yes	0%
South America									
Rio de Janeiro HIV	Brazil	Yes	Yes	Yes	No	Yes	429 (9%)	No	XX (8%
SobrHIV	Brazil	Yes	Yes	Yes	Yes	Yes	496 (10%)	No	XX (6%)
Asia									
YRG Care	India	No	Yes	No	Yes	Yes	104 (2%)	No	XX (8%)
HIV-NAT	Thailand	Yes	Yes	No	No	No	32 (<1%)	Yes	0%

Table 1: **Characteristics of antiretroviral treatment programmes in ART-LINC**

of whether the patient died, which cohort the were in, CD4 count, age, sex, and type of HAART regimen, **accounting appropriately for uncertainties in both indices and observation [A: please clarify what this means].**[10,11] Analyses were run on each of 20 datasets, including the input values, and the results combined with Rubin's rules.[11] We used random-effects Weibull regression models to estimate mortality hazard ratios accounting for heterogeneity between treatment programmes.[12,13] Models included both individual level (age, sex, baseline CD4 cell count, type of initial regimen, and stage of disease) and programme level characteristics (free of charge treatment, use of generic drugs, routine monitoring of virological response, tuberculosis clinic on site, and intensity of efforts to trace patients). We used a parametric bootstrap procedure with 300 bootstrap replications to derive confidence intervals for cumulative hazards at months 6 and 12. Finally, we compared mortality between low-income and high-income settings, adjusting for differences in age, sex, baseline CD4 cell count, type of initial regimen, and stage of disease. We used Stata software (version 9) for analyses. Results are presented as estimates of the probability of death, mortality rates, and hazard ratios (HRs) with 95% CIs.

Role of the funding source

The sponsors of the study had role in study design; the collection, analysis, or interpretation of data; the writing of the report; or the decision to submit the paper for publication.

Results

The ART-LINC dataset has 6498 treatment-naïve patients with a known date of starting HAART and at least one follow-up; 4810 (74%) patients also had a CD4 count at baseline, and were thus included in the analysis. Compared with treatment-naïve patients starting HAART without an immunological assessment, those with a documented baseline CD4 count were less likely to be male and more likely to be treated in publicly funded centres or programmes offering free care.[8] The characteristics of the programmes contributing data to the present analysis are shown in Table 1. Eligibility criteria for initiating HAART were generally advanced immunodeficiency or clinical disease. Six programmes were publicly funded through government and the remaining were run by non-governmental organisations or private doctors. All programmes included CD4 cell count monitoring, with measurements planned every 4–6 months. Routine monitoring of virological response was done in 10 programmes. 14 treatment programmes actively followed-up patients with telephone calls (often to mobile phones), letters, or home visits. 12 clinics provided free access to treatment. Costs to patients in the remaining six clinics varied from $8–198 per month for drugs, $15–33.50 per CD4 count, and $30–100 per viral load measurement. The number of patients included in this analysis ranged from 32 to 1056 patients, the proportion lost to follow-up from 0% to 44%.

	Low-income settings (n=4810)		High-income settings (n=22217)	
	n (%)*	Deaths	n (%)*	Deaths
Age (years)				
16–29	1013 (21%)	33	4125 (19%)	42
30–39	2188 (45%)	80	10421 (47%)	161
40–49	1177 (24%)	36	5043 (23%)	108
⩾50	432 (9%)	16	2628 (12%)	103
Median (IQR)	36 (30–42)	..	36 (31–43)	..
Sex				
Female	2461 (51%)	86	5486 (25%)	79
Male	2349 (49%)	79	16731 (75%)	335
Baseline CD4 (cells/μL)				
⩽25	917 (19%)	66	2081 (9%)	113
25–49	557 (12%)	25	1350 (6%)	58
50–99	805 (17%)	30	2181 (10%)	52
100–199	1217 (25%)	30	4038 (18%)	87
200–349	940 (20%)	10	6018 (27%)	66
⩾350	374 (8%)	4	6549 (30%)	38
Median (IQR)	108 (37–210)	..	234 (98–380)	..
Clinical stage				
CDC stage A/B, WHO stage I/II	867 (18%)	13	17142 (77%)	154
CDC stage C, WHO stage III/IV	1733 (36%)	97	5075 (23%)	260
Unknown	2210 (46%)	55	0 (0%)	..
Initial antiretroviral regimen				
NNRTI-based	3391 (70%)	120	5125 (23%)	71
Protease inhibitor-based	900 (19%)	30	13783 (62%)	276
Unknown or other combination	519 (11%)	15	3309 (15%)	67

*Data are n (%) unless otherwise indicated.

Table 2: **Baseline characteristics and mortality in first year of HAART treatment in low-income and high-income countries**

[A: please include percentages after the number of deaths, in this format n (xx%)]

The characteristics of the 12 cohorts from high-income countries (ART-CC) have been described elsewhere.[1] The database includes information on 22 217 patients who were followed-up in nine cohorts from Western Europe (including the multicentre EuroSIDA[18] study), two from Canada, and one from the USA. The cohorts and number of patients included in ART-CC are: French Hospital Database on HIV[14] (n=9167), AIDS Therapy Evaluation project Netherlands[15] (2720), Italian Cohort of Antiretroviral-Naïve Patients[16] (2203), Swiss HIV Cohort Study[17] (2141), EuroSIDA[18] (1144), Frankfurt cohort[19] (1031), Collaborations in HIV Outcomes Research US (CHORUS)[20] (981), Köln/Bonn Cohort[21] (759), Aquitaine Cohort[22] (649), Royal Free Hospital Cohort[23] (647), British Columbia Centre for Excellence in HIV/AIDS[2] (507), and South Alberta Clinic[24] (268). All cohorts follow-up patients actively with telephone or postal reminders, or both. Recording of deaths in ART-CC can be assumed to be near complete: in two cohorts, deaths are routinely ascertained from the national mortality register, and mortality rates in these two cohorts are similar to that in the others.

Patients from low-income countries were more likely to be female and more likely to start HAART with a NNRTI-based regimen (table 2). The proportion of participants who were women ranged from 29% in India to 79% at a site in South Africa; corresponding

[A: we do not use headings in the Results section]

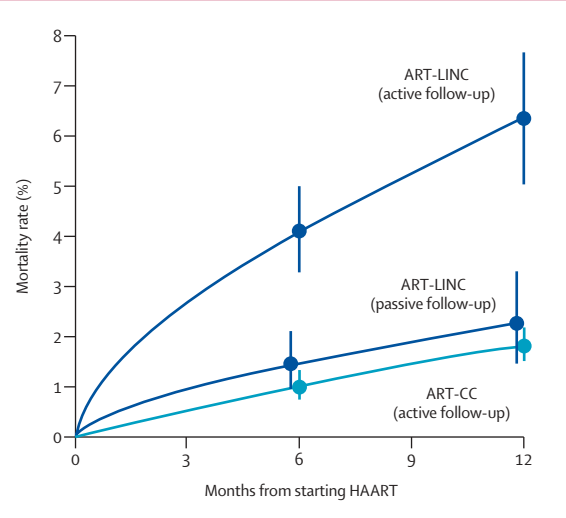

Figure 1: **Estimated cumulative probability of death in HAART programmes in low-income and high-income countries**
Vertical bars are 95% CIs.

proportions in high-income countries ranged from 9% (CHORUS, USA) to 31% (Swiss HIV Cohort Study). In patients from low-income settings, the median CD4 cell count at the time of starting HAART was 108 cells/μL (IQR 37–210) compared with 234 cells/μL (98–380) in patients from Europe and North America. Information on clinical stage was available for 2660 (54%) patients from low-income settings and for all patients from high-income settings. The route of infection was recorded in few patients in low-income countries, but most are assumed to have been infected through heterosexual intercourse. Conversely, in high-income cohorts, sex between men was the highest risk factor (8777 patients, 40%), followed by heterosexual sex (7910, 36%) and intravenous drug use (3553, 16%).

[A: The hazard ratio is much highere here for CD4 count =>350 compared with 200-349 – have the two been interposed by mistake?]

	Hazard ratio (95% CI)	
	Low-income settings (n=2725)	High-income settings (n=22217)
Age (years)		
16–29	1	1
30–39	1·12 (0·70–1·81)	1·16 (0·82–1·64)
40–49	1·09 (0·62–1·94)	1·47 (1·02–2·11)
⩾50	1·53 (0·74–3·18)	2·57 (1·78–3·71)
Sex		
Male	1	1
Female	0·84 (0·58–1·22)	0·85 (0·66–1·09)
Baseline CD4 (cells/μL)		
⩽25	1	1
25–49	0·77 (0·46–1·28)	0·83 (0·61–1·15)
50–99	0·54 (0·32–0·90)	0·55 (0·40–0·77)
100–199	0·37 (0·22–0·64)	0·67 (0·50–0·90)
200–349	0·14 (0·06–0·35)	0·44 (0·32–0·62)
⩾350	0·34 (0·12–1·01)	0·26 (0·17–0·39)
Clinical stage*		
CDC stage A/B, WHO stage I/II	1	1
CDC stage C, WHO stage III/IV	2·02 (1·02–4·02)	3·75 (2·96–4·74)
Initial regimen		
Two NRTIs+one NNRTI	1	1
Two NRTIs+one protease inhibitor	1·35 (0·76–2·40)	1·00 (0·77–1·31)
Unknown or other combination	1·13 (0·54–2·35)	1·17 (0·83–1·64)

*Entered for only 649 patients from low-income settings.

Table 3: **Hazard ratios of progression to death in HAART programmes in low-income and high-income countries**

In low-income settings, 2725 (57%) patients were treated in programmes with active follow-up procedures and 3029 (63%) had free access to HAART. Webtables 1 and 2 compare the characteristics of patients treated in programmes with active and passive follow-up, and those in programmes free of charge and those that were not free. 727 (15%) and 1104 patients (5%) were lost to follow-up during the first year of therapy in low-income and high-income settings, respectively. In low-income settings, loss to follow-up was 12% (331 of 2725) in programmes with active follow-up and 19% (396 of 2085) in programmes with passive follow-up; loss to follow-up occurred after a median of 5.8 months (IQR 2.5–7.7) and 3.4 months (1.4–6.9), respectively. In treatment programmes with active follow–up, those lost to follow-up and those followed-up at 1 year had similar baseline CD4 cell counts (median 115 cells/μL and 123 cells/μL), whereas patients lost to follow-up in programmes with no active follow-up procedures had considerably lower CD4 cell counts than those followed-up (median 64 cells/μL and 123 cells/μL). Webtable 3 compares the characteristics of patients lost to follow-up in both active and passive follow–up programmes.

After 6 months of treatment, CD4 and HIV-1 RNA measurements were available for 2789 (57%) and 2003 (48%) patients,respectively, in low-income settings, and in 19 560 (88%) and 19 164 (86%) in high-income settings. In low-income settings, patients with CD4 counts and viral load measurements at 6 months started HAART 1–2 years earlier, had higher CD4 cell counts at baseline, and were more likely to be treated in a programme providing free access to HAART than patients with no measurements. The median number of CD4 cells gained was 106 cells/μL (IQR 43–180) in low-income countries and 103 cells/μL (32–192) in high-income, countries. 1527 (76%) in low-income settings and 14825 (77%) in high-income countries had HIV-1 RNA levels lower than 500 copies/mL at 6 months.

At 1 year, mortality was estimated at 6.4% (95% CI 5.1–7.7) in low-income programmes with active follow-up (based on 124 deaths and 2236 person-years of follow-up), 2.3% (1.5–3.2) in low-income programmes with passive follow-up (41 deaths and 1508 person-years follow-up), and 1.8% (1.5–2.2) in programmes from high-income countries (414 deaths and 20 532 person-years follow-up) (figure 1). Treatment programmes with passive follow-up were excluded from subsequent analyses.

Baseline CD4 cell count was strongly prognostic both in low-income and high-income settings: the lower the baseline CD4 cell count, the higher the probability of death (table 3). Older age and more advanced clinical

stage were also associated with increased mortality. Among patients actively followed up, after imputation of missing information, an estimated 1608 patients (59.0%) had more advanced disease, whereas 504 patients (18.5%) had less advanced disease with a CD4 cell count above 200 cells/μL. There was little evidence for differences in progression rates between men and women or between patients starting HAART with different regimens.

In low-income settings, mortality was substantially higher in the first months after starting HAART than in later months. Mortality rates per 1000 person-years was 147 (95% CI 105–207) during month 1, 106 (71–160) in month 2, 51 (33–77) in months 3–4, 51 (33–79) in months 5–6, and 27 (19–40) in months 7–12 (figure 2). This trend was also noted in high-income settings, with mortality falling from 24 (21–27) during the first 6 months to 16 (14–19) during months 7–12. 97 (78%) of 124 deaths in low-income settings and 255 (62%) of 414 deaths in high-income countries happenedin the first 6 months **[A: ?rewording ok]**. Figure 2 shows crude and adjusted hazard ratios, comparing mortality in low-income countries to that in high-income settings, by time period after starting HAART. Adjusted hazard ratios fell from 4.31 (95% CI 1.57–11.81) during the first month on HAART to 1.48 (0.73–3.01) during the second half of the first year on HAART.

We assessed whether characteristics of treatment programmes in low-income settings affected outcome (table 4). In multivariable analysis, free access to treatment (with no costs to patients) was associated with lower mortality, whereas higher mortality was seen in programmes that included a tuberculosis clinic. There was little evidence for an association between mortality during the first year and the use of generic drugs or the routine monitoring of virological response.

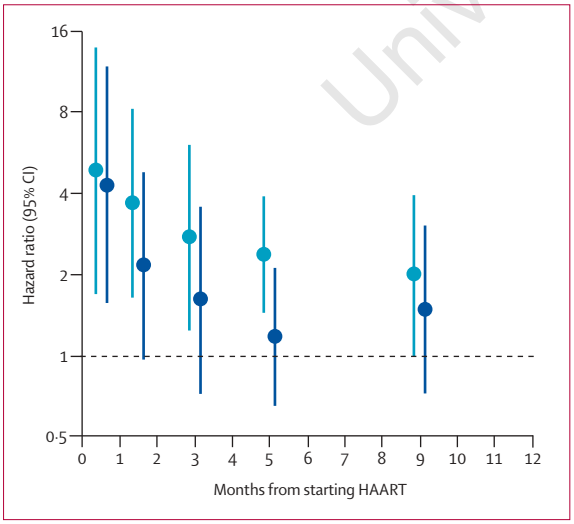

Figure 2: **Comparison of mortality in the months after starting HAART in low-income and high-income settings**
Shaded areas represent the periods for which hazard ratios were calculated. Vertical bars are 95% CIs.

	Hazard ratio (95% CI)	
	Unadjusted	Adjusted
Free access to treatment		
No	1	1
Yes	0·45 (0·13–1·54)	0·25 (0·08–0·78)
Use of generic drugs		
No	1	1
Yes	1·78 (0·52–6·13)	1·40 (0·45–4·39)
Routine monitoring of virological response		
No	1	1
Yes	1·43 (0·42–4·90)	2·28 (0·76–6·79)
Tuberculosis clinic on site		
No	1	1
Yes	2·42 (0·71–8·28)	3·76 (1·01–14·02)

Table 4: **Associations between HAART programme characteristics in low-income countries and mortality**

Discussion

Mortality rates of HIV-infected patients from low-income settings in Africa, South America, and Asia fell substantially within the first few months of HAART, and approached those seen in Western Europe and North America after 4–6 months. Patients in low-income settings started treatment with considerably more advanced immunodeficiency than those from industrialised countries, but virological and immunological response to HAART were similar in both settings, a finding that tallies with results from a recent collaborative analysis of treatment sites in the Asia and Pacific region,[26] a meta-analysis of the literature,[27] and reports from single centres.[28-30]

This systematic comparison of outcomes of HAART between low-income and high-income countries was done in treatment-naïve patients only, and results are therefore not confounded by previous antiretroviral therapy. Patients were enrolled in many different settings, which should reflect the experience with HAART in these regions. A substantial proportion of patients from low-income settings had to be excluded, and the data from low-income settings may thus be less generalisable than those from Europe and North America. Our study was restricted to adults, and results might not be applicable to infants and children. Information on AIDS events before starting treatment was missing for some patients in low-income settings. The statistical methods that we used to deal with this make use of the fact that previous AIDS events are strongly associated with measured factors at initiation, in particular, the CD4 cell count, while allowing appropriately for the additional uncertainty due to the missing data.[10,11]

Loss to follow-up is an important issue in treatment programmes in low-income settings. Ascertainment of deaths was clearly incomplete in ART-LINC clinics where no specific attempts were made to trace patients, confirming the results of a study from Côte d'Ivoire.[31] These sites were excluded from survival analyses. In treatment programmes that actively followed-up patients,

individuals lost to follow-up and those followed-up had similar baseline CD4 cell counts: those lost to follow-up were therefore probably not a selected group of patients with worse prognosis. All patients were started on HAART, which was documented for several months in most patients later lost to follow-up. This means that many will have survived the initial months, which have high mortality rates. Finally, our censoring strategy was conservative: follow-up was censored at the last visit. Clearly, some patients lost to follow-up will have died during the first year. In the worst-case scenario, if those lost to follow-up had the same prognosis as untreated patients, their baseline CD4 cell count (115 cells/μL) would mean that 25–50% died within 1 year.[32] This would increase mortality from 6.4% to as high as 15% **[A: ?is this what you meant]**. A study[33] of 910 adult treatment-naïve patients from Port-au-Prince, Haiti, reported that 13% died during the first year and 8% were lost to follow-up. This difference could suggest under-ascertainment of deaths in ART-LINC. Alternatively, the higher mortality in Port-au-Prince could be due to the more stringent criteria for initiating antiretroviral therapy (clinical AIDS or a CD4 cell count below 200 cells/μL). In ART-LINC, a substantial proportion of patients were free of AIDS at baseline, with a CD4 cell count above 200 cells/L. Notably, in both the Haitian study and ART-LINC about 80% of deaths occurred during the first 6 months.

The higher mortality in low-income countries during the first months of treatment compared with those in Europe and North America was only partly explained by the lower CD4 cell counts and more advanced clinical stage. Co-morbidities that are present in many patients starting HAART in resource-poor settings, including tuberculosis and invasive bacterial and fungal infections, might have increased mortality,[34] considering that access to prophylaxis, diagnostic facilities, and effective treatment for opportunistic infections is often limited. Immune reconstitution disease, an adverse consequence of restoration of pathogen-specific immune responses might also be a problem, particularly for tuberculosis.[35] Advanced immunodeficiency is associated with subclinical and disseminated infections, with high mycobacterial antigen load, and rapid improvement of restoration of immune function during HAART.[6,35] Inflammatory reactions occur in about one-third of co-infected patients receiving both HAART and tuberculosis treatment,[35,36] and might have contributed to the higher mortality we noted for sites with dedicated tuberculosis clinics.

However, even in the first months of HAART, mortality was lower than previously noted in untreated patients, suggesting an early beneficial effect of HAART. For example, compared with a mortality rate of 147 per 1000 person-years in the first month after treatment initiation in ART-LINC, mortality was 264 per 1000 person-years in the Cape Town AIDS Cohort[37] of 974 patients not treated with HAART, 353 per 1000 person-years in 746 untreated patients from the Gambia,[38] and 231 per 1000 person-years in a prospective study of 201 untreated Ugandan patients.[39]

Mortality was increased in programmes that charged fees. The World Bank, the International Monetary Fund, and other agencies have been criticised for promoting the privatisation of health services and private financing of health services through user fees.[40,41] Whitehead and colleagues[42] have argued that market-oriented policies can result in untreated morbidity, reduced and more unequal access to care, further impoverishment of those who are already poor, and can promote the irrational**[A: ?do you mean 'inappropriate' rather than 'irrational']** use of drugs. For example, treatment might have been stopped when money ran out: payments for antiretrovirals during the initial phase of therapy do not mean that households have the ability to pay for extended periods of time. In these settings, treatment might also be more likely to be interrupted and resumed upon clinical deterioration, which could promote viral resistance to drugs. Our results extend those from a systematic review and meta-analysis,[27] which recorded that provision of HAART free of charge to the patient was associated with an increased probability of achieving and maintaining suppression of viral replication. Although we acknowledge that other aspects of the delivery of care could have confounded associations, there must be concern that the "inverse equity hypothesis", which stipulates that health inequities will get worse as effective new public health interventions initially reach those of higher socioeconomic status and only later the poor, could be borne out in the case of HAART in some resource-poor settings.[43,44]

In many of the countries included in our study, access to potent antiretrovirals continue to be limited. For example, the WHO estimates that as of June, 2005, the percentage of people in need of antiretroviral therapy who received HAART was 4–8% in Nigeria, 4–9% in India, 10–17%% in Côte d'Ivoire, 10–14% in South Africa, 11–14% in Malawi, 12–17% in Kenya, 11–19% in Cameroon, and 35–43% in Uganda.[4] Thailand, Botswana, and Brazil are providing treatment to half or more of people living with HIV/AIDS that need it, consistent with the WHO "3 by 5" target.[4] Clearly, the global health emergency that was declared by the United Nations General Assembly in 2001 continues.

Effective antiretroviral therapy is feasible in low-income settings, but, compared with industrialised countries, mortality is high in the first months. Eligibility for antiretroviral treatment and the need for treatment of tuberculosis should be determined earlier, and HAART should be started before serious co-morbidities develop. The scalingup of HAART should therefore be accompanied by an expansion of voluntary counselling and testing services, and by efforts to reduce the stigma and adverse social effects associated with a positive HIV test, which might encourage more people at risk of HIV to seek testing **[A: ?ok]**.

Contributors
F Dabis, M Egger, and M Schechter conceived the ART-LINC Collaboration and wrote the first draft of the study protocol. All collaborators contributed to the final version of the protocol. M Egger conceived and coordinated the current analyses. M Brinkhof, M May, and J Sterne did statistical analyses. M Egger and P Braitstein wrote the first draft of the paper; all authors contributed to the final text. All investigators assisted in implementation, fieldwork, or data collection at study sites.

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Conflict of interest statement
Add in from faxes

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Determinants of Mortality and Nondeath Losses from an Antiretroviral Treatment Service in South Africa: Implications for Program Evaluation

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(See the editorial commentary by Weller on pages 777–8)

Background. The scale-up of antiretroviral treatment (ART) services in resource-limited settings requires a programmatic model to deliver care to large numbers of people. Understanding the determinants of key outcome measures—including death and nondeath losses—would assist in program evaluation and development.

Methods. Between September 2002 and August 2005, all in-program (pretreatment and on-treatment) deaths and nondeath losses were prospectively ascertained among treatment-naive adults ($n = 1235$) who were enrolled in a community-based ART program in South Africa.

Results. At study censorship, 927 patients had initiated ART after a median of 34 days after enrollment in the program. One hundred twenty-one (9.8%) patients died. Mortality rates were 33.3 (95% CI, 25.5–43.0), 19.1 (95% CI, 14.4–25.2), and 2.9 (95% CI, 1.8–4.8) deaths/100 person-years in the pretreatment interval, during the first 4 months of ART (early deaths), and after 4 months of ART (late deaths), respectively. Pretreatment and early treatment deaths together accounted for 87% of deaths, and were independently associated with advanced immunodeficiency at enrollment. Late deaths were comparatively few and were only associated with the response to ART at 4 months. Nondeath program losses (loss to follow-up, 2.3%; transfer-out, 1.9%; relocation, 0.7%) were not associated with immune status and were evenly distributed during the study period.

Conclusions. Loss to follow-up and late mortality rates were low, reflecting good cohort retention and treatment response. However, the extremely high pretreatment and early mortality rates indicate that patients are enrolling in ART programs with far too advanced immunodeficiency. Causes of late access to the ART program, such as delays in health care access, health system delays, or inappropriate treatment criteria, need to be addressed.

Although sub-Saharan Africa is home to just 10% of the world's population, more than 60% of the world's HIV-infected people live there; in 2005 alone, an estimated 2.4 million people in the region died of HIV/AIDS [1]. As one component of a strategy to address this devastating epidemic, access to antiretroviral treatment (ART) is now being rapidly expanded within the region. In June 2005, it was estimated that 6.5 million people urgently required treatment in resource-limited settings. In view of the enormous scale of this intervention, a simplified programmatic approach has been adopted to facilitate delivery of treatment [2–4]. The efficacy of ART, as reflected by virological and immunological responses, is similar among patients treated in high-income countries and patients treated in resource-limited countries [5, 6]. The impact of ART programs in low-income countries is, therefore, unlikely to be related to questions of drug efficacy, but rather to health system issues and program effectiveness [7]. Parameters with which to evaluate the effectiveness of programs need to be identified. Tuberculosis treatment programs provide a useful model of how evaluation of carefully defined outcome measures permits

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longitudinal program assessments and comparisons [8]. Similar principals might usefully be applied to ART programs.

Two key goals of ART programs are to prevent mortality and to retain people within the program, receiving treatment in the long term. In this study, we describe mortality occurring (1) in the short interval between program enrollment and initiation of ART (the pretreatment interval), (2) during early ART (0–4 months), and (3) during late ART (4 months to 3 years). We also characterized nondeath losses. We have accurately quantified these different losses during the first 3 years of a community-based ART program in South Africa. We identified the temporal distribution and determinants of these different outcomes, which have thereby provided important insights into program performance.

SUBJECTS AND METHODS

ART. The ART service described here is based at the Gugulethu Community Health Centre in Cape Town and has previously been described in detail [9–11]. This district has a predominantly African population of >300,000, the vast majority of whom live in conditions of low socioeconomic status. In 2003, the antenatal HIV seroprevalence was 28%. Patients are referred to the ART program from primary care HIV clinics. Treatment criteria are based on the World Health Organization's (WHO's) 2002 recommendations [3], which include a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count <200 cells/μL.

The time between enrollment of a patient in the service and initiation of ART is ~1 month, to permit thorough evaluation of patients and preparation for treatment as described previously [9–11]. Assignment of each patient to a community-based therapeutic counselor facilitates this preparation and provides an efficient system for determining outcomes for all patients, including tracing those who do not attend follow-up appointments. A proportion of patients do not initiate treatment in this service for a variety of reasons other than death; such individuals are deferred from the program, and follow-up is censored at this time-point. However, all individuals contributed to the pretreatment person-time of observation, regardless of whether they subsequently received ART.

First-line ART consisted of stavudine and lamivudine plus a nonnucleoside reverse transcriptase inhibitor (efavirenz or nevirapine). The second-line regimen for those for whom first-line treatment failed was composed of lopinavir/ritonavir, zidovudine, and didanosine. All treatment was provided free of charge. Treatment adherence and viral load suppression at a level of <400 copies/mL in this cohort both exceeded 90% at 1 year [10–12]. All patients with CD4 counts <200 cells/μL received prophylaxis with daily trimethoprim-sulfamethoxazole or dapsone as an alternative. In addition to scheduled clinic appointments at 4, 8, and 16 weeks and once every 16 weeks thereafter, patients had open access to the clinic for medical problems.

Definitions. "Permanent deferrals" were patients who did not receive ART for a reason other than pretreatment death and who were subsequently excluded from the program. "Pretreatment deaths" were those that occurred among patients who had enrolled into the program but who had not yet initiated ART. "Early on-treatment deaths" were those that occurred during the first 4 months of ART, and "late on-treatment deaths" were those that occurred after 4 months of ART. "Transfers-out" were patients whose care was transferred to another ART program. "Relocations" were patients who moved to another location but who were not referred to an ART service in the new area. "Losses to follow-up" were patients receiving ART who were >4 weeks late for a scheduled clinic or pharmacy visit and who were neither transfers-out nor relocations. "Nondeath losses" were the sum total of transfers-out, relocations, and losses to follow-up.

Data sources. Structured clinical records were maintained for all patients screened on entry to the ART program. This information was transferred to a computer database on a weekly basis. Data were analyzed from the start of the program in September 2002 until data censorship in August 2005. This study was approved by the Research Ethics Committee of the University of Cape Town, and all patients who were enrolled gave written, informed consent.

Data analysis. Data were analysed using Stata, version 9.0 (StataCorp). Wilcoxon rank-sum and Fisher's exact tests were used to compare medians and proportions, respectively. In separate analyses, we calculated rates of mortality and other outcomes from either program enrollment (the date of initial screening by the service) or from ART initiation. Person-time was censored at the end of August 2005 for individuals who were alive and who had been retained by the service. Product-limit analyses were used to calculate the instantaneous hazard of death or other losses through time among individuals receiving ART; we plotted smoothed hazard-function estimators using weighted kernel-density estimates based on an Epanechnikov function [13]. In other product-limit analyses, log-rank tests were used to examine the effect of baseline WHO clinical stage and category of CD4 cell count on survival probabilities. All statistical tests are 2-sided at $\alpha = .05$.

Multivariate analysis employed proportional hazard models to examine determinants of mortality among individuals receiving ART. Separate models were developed to examine factors associated with early mortality, late mortality, and all deaths. In separate models, baseline CD4 cell count was modeled as both a continuous variable (per 50-cell/μL change in the CD4 cell count) and a categorical variable, to demonstrate

threshold effects. Covariates were included in the model if they demonstrated an appreciable association with the relative hazard of mortality, or if their removal affected associations involving other covariates. Model diagnostics and the proportional hazards assumption were examined using Schoenfield and scaled Schoenfield residuals [14].

RESULTS

Cohort and follow-up. During the period of September 2002 through August 2005, 1340 patients enrolled in the program. Those who were not naive to ART ($n = 53$) and those <15 years of age at enrollment ($n = 52$) were excluded. Among 1235 patients who remained in the analysis, the median age was 33 years (interquartile range [IQR], 28–38 years), and 882 patients (71%) were female. Baseline plasma viral load and blood CD4 cell counts were available for 1086 and 1120 patients, respectively. The median plasma viral load was 4.81 $\log_{10}$ copies/mL (IQR, 4.42–5.23 $\log_{10}$ copies/mL), and the median blood CD4 cell count was 100 cells/μL (IQR, 47–160 cells/μL). Most patients (79%) had symptomatic disease, and 644 (52%) and 332 (27%) had WHO stage 3 and 4 disease, respectively.

Nine hundred twenty-seven patients (75%) received ART during the study period, and 117 (9.5%) were preparing for treatment at the time the study was censored (figure 1). The median time between enrollment in the program and initiation of treatment was 34 days (IQR, 28–50 days). Over the course of the study, 170 person-years of observation were accrued in the pretreatment interval, and 808 person-years were accrued during treatment.

Numbers and characteristics of deaths and nondeath losses. One hundred thirty-five (9.5%) patients were deferred from the service before initiation of ART (figure 1) for a variety of reasons, including decision to access treatment elsewhere, failure to attend follow-up clinic appointments, movement out of the area, or for psychosocial reasons. Among total deaths ($n = 121$), 56 (46%) occurred in the pretreatment interval, 49 (40%) were early on-treatment deaths, and 16 (13%) were late on-treatment deaths. The death rate was high in the pretreatment interval (33.3 deaths/100 person-years; 95% CI, 25.5–43.0 deaths/100 person-years) but decreased during the first 4 months of ART (19.1 deaths/100 person-years; 95% CI, 14.4–25.2 deaths/100 person-years), and was lower still beyond 4 months ART (2.9 deaths/100 person-years; 95% CI, 1.8–4.8 deaths/100 person-years). After 1 year of ART, the mortality rate was just 1.3 deaths/100 person-years (95% CI 0.4–3.9 deaths/100 person-years).

Among those who initiated ART ($n = 927$), 110 patients (11.9%) were lost from the program (figure 1). Among these, death was the most common reason, accounting for 65 (7.0%) of losses, whereas 45 (4.9%) were due to other causes. These

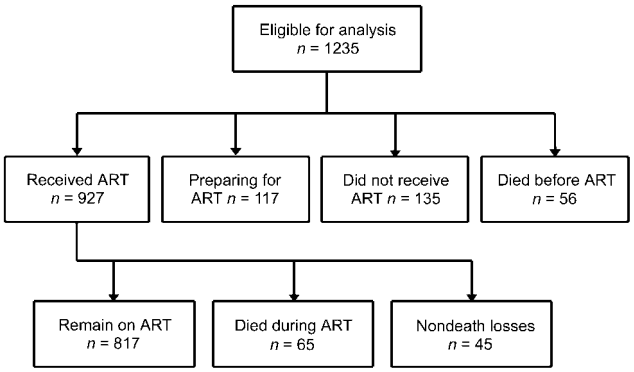

Figure 1. Flow diagram showing the outcomes of patients included in the analysis ($n = 1235$) at the time that data were censored. ART, antiretroviral therapy.

nondeath losses were due to transfer-out (18 [1.9%]), relocation (6 [0.7%]), and loss to follow-up (21 [2.3%]).

We compared the characteristics of patients who were lost from the program due to death with those who were lost due to other reasons or who continued to receive treatment when the data was censored (table 1). In univariate analyses, those who died in the pretreatment interval or during early ART were more likely to have a prior AIDS diagnosis, a baseline CD4 cell count <100 cells/μL, and a baseline plasma viral load $>10^5$ copies/mL, compared with those who remained in the program. Those who died after 4 months of ART (late deaths) were also more likely to have a baseline CD4 cell count <100 cells/μL, although this association did not persist in the multivariate analysis (see below). In contrast, nondeath losses were not associated with baseline immunodeficiency. Kaplan-Meier analyses confirmed that the probability of in-program death was strongly associated with WHO stage of disease and baseline CD4 cell count (figure 2).

Temporal distribution of program losses. The risk of loss from the program changed markedly during follow-up of patients receiving ART (figure 3*A*). Risk of death had 3 distinct phases: an initially high—but steeply decreasing—risk during the initial months of ART, followed by a moderate risk of death up to ~1 year, and a very low risk of death after 1 year (figure 3*B*). In contrast, the risk of program loss due to other causes was relatively constant (figure 3*C*). After ~1 year of ART, risk of nondeath losses to the program exceeded losses due to death.

Multivariate analysis for risk of death during ART. In multivariate analysis to predict the relative hazards of death during ART, death was significantly associated with baseline CD4 cell count and WHO clinical stage, but not with age, sex, or baseline viral load. However, risk factors for early deaths versus late deaths differed markedly (table 2). Early on-treatment deaths were associated with advanced WHO clinical stage, lower baseline blood CD4 cell counts, and male sex (table 2).

In contrast, late on-treatment deaths were only independently associated with the response to ART at 4 months, as reflected by blood CD4 cell count and viral load. Although the number of late deaths was small (data were available for 12 of 16 deaths), this association with CD4 cell count was statistically highly significant, and the trend towards an association with viral load approached statistical significance.

CD4 cell count increases and risk of loss to program. We examined how on-treatment program losses (death and nondeath) were associated with CD4 cell counts at baseline and after 4 months of ART. Although the median CD4 cell increases among patients retained in the cohort (99 cells/μL; IQR, 49–162 cells/μL) were similar to those among nondeath losses (83 cells/μL; IQR, 49–133 cells/μL), those who subsequently died had much smaller CD4 cell count increases (44 cells/μL; IQR, 5–83 cells/μL). The vast majority of deaths occurred among individuals who had a baseline CD4 cell count <100 cells/μL and a CD4 cell count <200 cells/μL at 4 months (figure 4*A*). In contrast, nondeath losses were not associated with the CD4 cell count distribution (figure 4*B*).

DISCUSSION

In this study we carefully quantified mortality and nondeath losses in a community-based ART program in South Africa and identified the temporal distribution and risk factors associated with these losses. We defined pretreatment, early and late ART mortality, and nondeath losses as useful outcome measures of ART programs. Loss to follow-up and late mortality rates were low, reflecting excellent cohort retention and treatment response in this program. In contrast, however, pretreatment and early mortality rates were very high: this finding very strongly suggests that patients were enrolling with far too advanced immunodeficiency. To reduce in-program mortality, the causes of late program entry need to be addressed.

On-treatment mortality rates were similar to or better than those previously reported from resource-limited settings [15–19]. Although previous studies have not reported mortality occurring within the program prior to actual initiation of ART, we demonstrated that a large proportion of early program

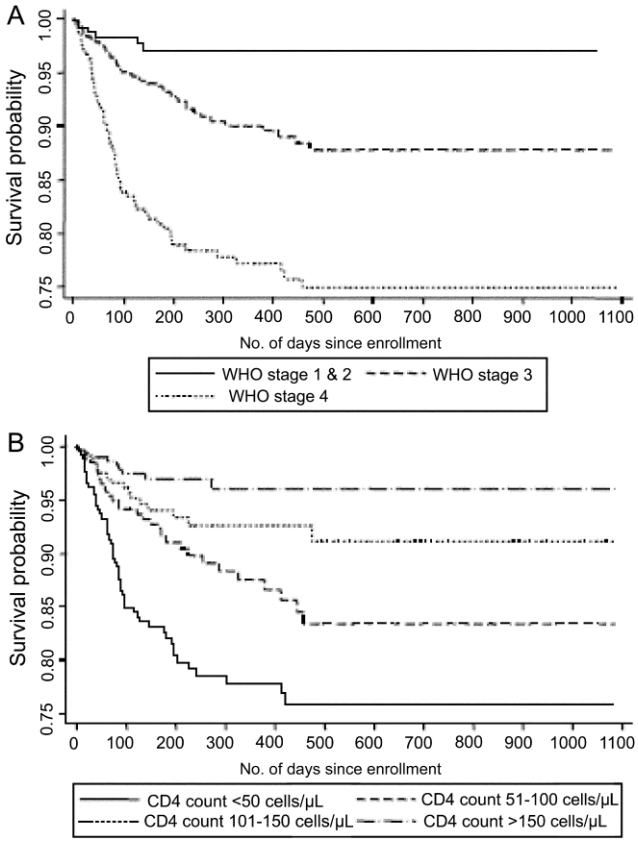

Figure 2. Kaplan-Meier plots showing survival of patients from entry into the program stratified by World Health Organization (WHO) clinical stage *(A)* and baseline blood CD4 cell count *(B)*. These reveal that risk of death early in the program was strongly associated with the baseline degree of immunodeficiency at enrollment.

Table 1. Baseline characteristics of patients who either remained in the program, died, or were lost from the program for other reasons (transfer-out, relocation, or loss to follow-up).

Characteristic	Remained in program	Death			Other program losses
		Pretreatment	Early	Late	
No. of patients	817	56	49	16	45
Female sex	601 (74)	37 (66)	25 (49)	12 (75)	32 (71)
Age, median years (IQR)	33 (28–38)	32 (28–40)	34 (28–39)	35 (32–41)	31 (27–35)
Prior AIDS diagnosis	207 (25)	30 (54)[a]	29 (59)[a]	7 (44)	12 (27)
CD4 count <100 cells/μL	410 (50)	35 (63)[a]	40 (82)[a]	13 (81)[b]	27 (60)
Viral load >10^5 copies/mL	301 (37)	19 (59)[b]	26 (54)[b]	9 (56)	21 (47)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Statistical comparisons were made between characteristics of those who were retained in the program, compared with the characteristics of those who were lost to the program. IQR, interquartile range.

[a] $P < .05$.
[b] $P < .001$.

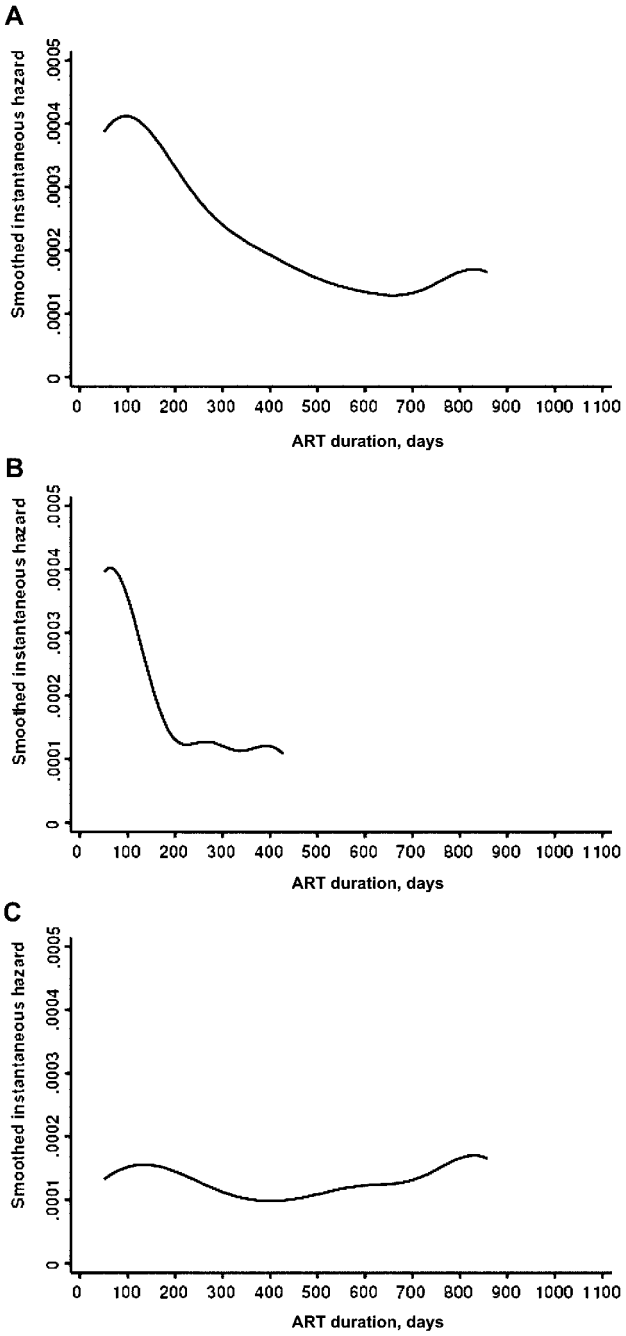

Figure 3. Smoothed hazard estimates for total losses to program *(A)*, death *(B)*, and nondeath losses to program *(C;* i.e., transfers-out, relocations, and losses to follow-up). Risk of mortality was initially high but decreased steeply, and there were very few deaths after 400 days of ART. Risk of nondeath losses was relatively consistent during follow-up. ART, antiretroviral therapy

deaths occur during this pretreatment interval [9]. The pretreatment interval in the present analysis (median, 34 days) is shorter than reported elsewhere [15] but permitted careful evaluation, investigation, and treatment of opportunistic infections, as well as thorough preparation of patients for ART. We believe this preparation is key to the very high adherence rates and excellent virological and immunological outcomes observed in this program [10, 11]. Within this service, clinicians were able to "fast-track" patients who had the most advanced immunodeficiency; however, shortening the pretreatment interval for all patients may not necessarily reduce early mortality and may actually compromise long-term outcomes.

The present analysis shows that, after 3 years of this program, deaths in the pretreatment interval contributed >46% of total program mortality and therefore represent a very important outcome measure. This mortality is likely to reflect a far greater burden of mortality that is actually occurring prior to program entry. Those who died in the first 4 months of treatment (early deaths) shared the same baseline characteristics and risk factors as those who died in the pretreatment interval (tables 1 and 2); together, deaths in these 2 intervals constituted 87% of total program mortality. The strong association of these deaths of patients who had advanced immunodeficiency at baseline clearly indicates that many of these patients had advanced disease that could not be salvaged by ART, despite the active management in many patients of concurrent infections. One-half of the patients enrolling into this program had a baseline CD4 cell count <100 cells/μL. Kaplan-Meier survival analyses revealed that early mortality was high among patients with WHO stage 3 and stage 4 disease (i.e., symptomatic disease) and patients with baseline CD4 cell counts <100 cells/μL (figure 2).

The reasons why patients typically enter this and other programs in resource-limited settings with such advanced disease

Table 2. Results of separate Cox's models predicting relative hazards of early deaths ($n = 49$) and late deaths ($n = 12$) after initiation of antiretroviral therapy (ART).

Variable	Early deaths	Late deaths[a]
Age[b]	1.01 (0.97–1.05)	1.07 (0.99–1.14)
Sex		
Female	1.00	1.00
Male	2.00 (1.10–3.62)	0.58 (0.16–2.03)
WHO stage		
1, 2, or 3	1.00	1.00
4	2.78 (1.52–5.09)	1.71 (0.50–5.82)
Baseline CD4 count[c]	0.62 (0.47–0.83)	0.98 (0.58–1.65)
CD4 count at 4 months	…	0.42 (0.25–0.73)
Baseline viral load		
$\leqslant 10^5$ $\log_{10}$ copies/mL	1.00	1.00
$>10^5$ $\log_{10}$ copies/mL	1.37 (0.77–4.44)	1.24 (0.36–4.28)
Viral load at 4 months		
<50 copies/mL	…	1.00
$\geqslant$50 copies/mL	…	3.17 (0.94–10.71)

NOTE. Data are hazard ratio (95% CI). WHO, World Health Organization.
[a] Complete data available for 12 of 16 patients.
[b] Analyzed as a continuous variable.
[c] Analyzed in 50-cell/μL increments.

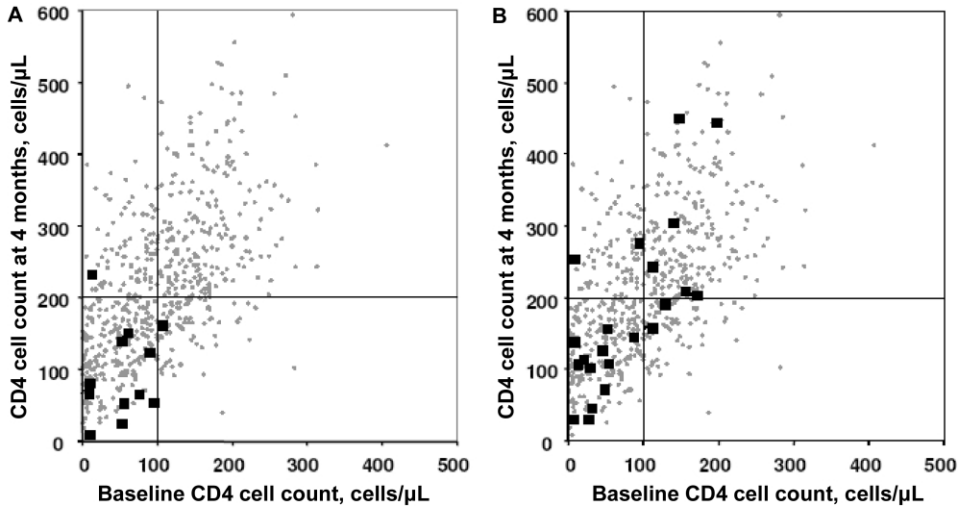

Figure 4. Scatter plots of baseline CD4 cell count against CD4 cell count at the 16-week follow-up time-point. On these plots, individual patients *(dark squares)* who either died *(A;* data available for 12 of 16 patients) or were lost to the program for other reasons *(B; n* = 22) after 4 months of antiretroviral therapy (ART) are indicated. Deaths—but not nondeath losses—occurred among individuals with advanced baseline immunodeficiency and persisting immunodeficiency after 16 weeks of ART.

need to be identified. Possible reasons include (1) barriers to voluntary counseling and testing and to access to health care, (2) lack of routine blood CD4 cell count testing for patients with new HIV diagnoses, (3) health system delays in referral patients to ART clinics, (4) waiting times to enter programs, and (5) criteria for initiation of ART that only include patients with advanced disease. How to best promote early access of ART-eligible patients to treatment programs is a central challenge facing the scale-up of these programs in resource-limited settings [20]. Moreover, the South African national ART program uses the WHO 2002 ART guidelines, which restricts treatment to patients with stage 4 disease or CD4 cell counts <200 cells/μL [3]. The revised WHO 2003 guidelines for resource-limited settings recommend earlier treatment [4], and the use of these may help to lower mortality. Moves to earlier initiation of treatment are supported by a collaborative analysis of datasets that reveal that mortality rates in ART programs in resource-limited settings are higher than those in high-income countries [5].

In contrast to early deaths, mortality after 4 months of ART (late death) was independent of baseline immune status but was strongly associated with the response to ART, as reflected by the absolute blood CD4 cell count and viral load at 4 months. As such, the late mortality rate reflected therapeutic success, including drug regimen efficacy and tolerability as well as patient adherence to treatment. After the first year of ART, mortality rate was very low—approaching 1% per year—and the risk of loss to program due to nondeath causes exceeded those due to death (figure 3). Thus, the effectiveness of programs beyond 1 year is likely to relate to issues of long-term patient retention rather than death. As patients remain healthy on long-term medication, their motivation to continue treatment in the longer term may diminish [21]. It remains unknown whether an increasing rate of treatment failure and a secondary increase in the death rate may occur with longer follow-up.

Among ART programs in sub-Saharan Africa, rates of nondeath program losses range from <5% [15, 17] to >50% [22]. When patients are termed "lost to follow-up" simply on the basis of persistent failure to attend clinic appointments, it is possible that some may in fact have died without detection by the ART program. The true mortality rate among patients receiving ART may, therefore, often be underestimated. However, in this analysis, the use of community-based therapeutic counselors that were allocated to each patient enhanced the data completeness and assessment of outcomes for patients who failed to attend follow-up appointments.

Nondeath program losses in this setting were heterogeneous in nature. Patients who moved out of the area accounted for 22% of total on-treatment program losses. As opposed to those transferred to another ART program, the care of some patients (here termed "relocations") was not transferred, often because of lack of provision of ART services in other areas. Because the number of patients who are physically well and who are receiving long-term medication is increasing, the number of patients moving out of an area for social or economic reasons may continue to increase. This finding emphasizes the importance of systems within national ART programs that can ensure continuity of care for highly mobile populations of young adults. Losses to follow-up in this treatment service were low, likely as a result of dedicated community-based counsellors allocated to each patient and thorough preparation of patients for ART.

In summary, high pretreatment and early on-treatment mortality rates in this program reflected very advanced immunodeficiency. The reasons for patients' late access to the program urgently need to be identified. It is likely that these early in-program deaths reflect a far greater burden of mortality within the health system and in the community that is occurring "up stream" of the ART program. However, this study found that, once patients have initiated ART and survived the initial few months of treatment, the risk of death or loss to the program thereafter was very low. Evaluation of these various outcome measures provides important means of assessment for ART programs, which thereby facilitates development of optimum models of care in resource-limited settings.

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Potential conflicts of interest. All authors: no conflicts.

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Early antiretroviral therapy mortality in resource-limited settings: what can we do about it?

Linda-Gail Bekker[a,b], Matthias Egger[c] and Robin Wood[a]

Purpose of review

Highly active antiretroviral therapy has markedly reduced HIV morbidity and mortality in industrialized countries. Expanded access to the 6.5 million individuals in immediate need of antiretroviral therapy using a public-health-systems approach is now promulgated as an international policy. An approximate 1.6 million individuals have already accessed antiretroviral therapy within programs in resource-poor settings.

Recent findings

Early studies from these treatment programs confirm similar virologic and immunologic responses to antiretroviral therapy as were observed earlier in industrialized settings. While medium-term reductions in morbidity and mortality also parallel those reported from Europe and North America, of particular concern is the observation that mortality immediately after starting antiretroviral therapy in resource-poor settings is several-fold higher than that of similar patients initiating antiretroviral therapy in industrialized settings.

Summary

This early mortality is multifactorial and is both a reflection of a very high preantiretroviral therapy mortality and a variety of factors such as comorbid conditions, late presentation, immune restoration disease, together with limited treatment and diagnostic options. Causes of mortality immediately prior to and during early antiretroviral therapy are reviewed and strategies to reduce mortality are identified and discussed.

Keywords

early mortality, resource-limited ART programs

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Abbreviations

ART	antiretroviral therapy
ARV	antiretrovirus
IRD	immune restoration disease
VCT	Voluntary Counseling and Testing

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Introduction

The increasingly widespread use of highly active antiretroviral therapy (ART) since 1996 has substantially improved the prognosis of HIV-infected patients [1–3] and an estimated 3 million years of life have been saved as a result of access to ART in the USA alone [4••].

Since the launch of the '3 by 5' initiative of the WHO in December 2003, 1.6 million individuals had accessed ART worldwide by the middle of 2006 [5]. A substantial number of deaths have been averted in low and middle-income countries as a result of widened access to HIV treatment [6]. Only 24% of those in need have received ART, however, and there remain an estimated 6.5 million people in urgent need of ART [5].

ART is now considered an integral part of the comprehensive approach to HIV prevention, care and support [7] and there is a global commitment to universal access to ART for all who need it by the end of 2010 [8].

The public-health delivery of ART focuses on maximizing survival at the population level through standardized sequencing of limited drug regimens, delivered by means of simplified approaches and supported by clinical and basic laboratory monitoring. ART delivery has been expanded massively in Zambia and Malawi using a public health approach [9,10]. While virologic and immunologic responses to ART are comparable in poor and rich-resourced settings [11••,12], mortality in the first month after initiating ART is over four-fold higher in resource-poor countries [11••,13]. With the scaling up of treatment in low and middle-income countries, there is opportunity to better understand causes of mortality and an opportunity to reassess and optimize program strategies. We review published data that describe patterns of mortality and identify possible strategies to reduce early mortality in low-income ART programs.

Causes of mortality before antiretroviral therapy

The primary rationale for an ART program is to decrease general-population HIV morbidity and mortality. Since the beginning of the ART era, antiretrovirals (ARVs) have been recognized in industrialized settings to decrease the incidence of new AIDS-related conditions [1–3] but it is only recently that ART has been recognized to also decrease non-AIDS-related complications

Table 1 Causes of mortality pre-antiretroviral therapy

	Industrialized settings	Resource-poor settings
Early HIV infection	Diseases of affluence Hepatitis B/C coinfection	Malnutrition Malaria Gastrointestinal infections
Advanced HIV infection	Opportunistic diseases PCP, MAI, CMV, KS	Tuberculosis Wasting syndrome Lower respiratory tract infections

CMV, cytomegalovirus; KS, Kaposi's sarcoma; MAI, *Mycobacterium avium intracellulare*; PCP, *Pneumocystis carinii* pneumonia.

such as cardiovascular disease and non-AIDS-related neoplasms which occur at higher CD4 cell counts [14••]. In resource-poor settings, significant mortality also occurs at higher CD4 cell counts. In a South African cohort [15•] followed before access to ART, 50% of deaths occurred before confirmation of an AIDS diagnosis. Rates of death and progression to AIDS for those with CD4 cell counts between 200 and 350 cells/μl were also higher than those reported in European cohorts [15•]. Following development of AIDS, the median survival of untreated patients in South Africa and rural Uganda is just 9–10 months [16,17] compared with around 2 years in high-income countries prior to the advent of ART [18–20].

Currently, the majority of patients access ART in sub-Saharan Africa with low CD4 cell counts and advanced clinical stage [9,10,11••,12,13]. Inevitable delays in accessing and initiating ART result in significant programmatic mortality [13]. The major causes of mortality in early and advanced HIV infection for both industrialized and resource-poor settings are shown in Table 1.

Differences between antiretroviral therapy programs in industrialized and resource-poor settings

Some of the differences in ART programs in well and less well resourced settings are summarized in Table 2. The scaling-up of antiretroviral therapy in resource-poor settings has utilized a public-health approach [7,21]. WHO guidelines make provision for two treatment regimens: an initial nonnucleoside reverse transcriptase enzyme inhibitor (NNRTI)-based regimen followed by a protease inhibitor-based regimen. The public health approach is very different from the practice in Europe and North America, where individualized therapy has led to utilization of a much greater array of ART combinations. An analysis conducted between 2003 and 2005 showed that 47 and 59 regimens were used to treat 90% of patients in Western Europe and the USA, respectively. In contrast, in Africa and Asia, only three regimens were used to treat 90% of patients [22].

Table 2 Characteristics of antiretroviral therapy programmes in industrialized and resource-poor settings

Industrialized settings	Resource-poor settings
Individualized management	Public-health approach
HIV epidemic predominantly urban	HIV epidemic urban and rural
Clinic-based treatment	Clinic and community-based treatment
High proportion aware of HIV status	Low proportion aware of HIV status
High ART coverage	24% of patients in need of ART on ART
Guidelines for early ART initiation	Guidelines for late ART initiation
Wide choice of ART options	Limited number of ART regimens
Frequent viral and CD4 count monitoring	Infrequent monitoring

ART, antiretroviral therapy.

Fifty-five percent of the world's population is rural, whereas, in industrialized countries, approximately 75% are urbanized. The HIV epidemic mirrors this distribution and is predominantly urban in North America and Europe and both rural and urban in resource-poor settings [23]. Approximately 1.0–1.2 million persons are estimated to be living with HIV in the USA, of whom 25% are unaware of their infection and likely to have transmitted their infection unknowingly [24,25]. In sub-Saharan Africa, 24.5 million individuals are living with HIV [26], but fewer than 10% are aware of their HIV status [27].

Despite global efforts to expand ART access as of December 2005, fewer than 80% of individuals in need of ART were receiving ART. Coverage in Europe and North America was between 75 and 100%, whereas in many countries of sub-Saharan Africa, coverage in 2005 was less than 10% of need [28].

The WHO guidelines [21] for scaling up ART in resource-poor settings were first published in 2002 and recommended initiation of ART for those with AIDS or a CD4 count of less than 200 cells/μl or an AIDS diagnosis. More recent revisions have allowed for earlier treatment of those with non-AIDS symptoms and CD4 cell counts of less than 350 cells/μl [7]. North American and European guidelines recommend therapy for all symptomatic individuals and those with CD4 cell counts of less than 350 cells/μl [29,30].

Virologic monitoring of ART is frequently not available in resource-poor settings because of high cost and lack of appropriate infrastructure and, when available, is less frequently performed than in industrialized settings [22,31].

Causes of mortality on antiretroviral therapy: mortality during early antiretroviral therapy

The efficacy of ART as measured by viral suppression appears similar in resource-rich and resource-poor settings [11••,12,32–37], but early deaths on ART are more

frequent in resource-poor settings [11••,13]. Early deaths occur predominantly in those with advanced disease, with risk factors for death being CD4 counts of less than 100 cells/μl, a prior AIDS diagnosis and older age [11••,13]. The higher mortality in low-income countries during the first months of treatment compared with those in Europe and North America is only partly explained by the lower CD4 cell counts and more advanced clinical stage [11••,13]. The mortality occurring immediately after starting ART appears to reflect the high mortality preceding commencement of ART. Comorbidities that are present in many patients starting ART in resource-poor settings, including tuberculosis and invasive bacterial and fungal infections, may increase mortality, particularly as access to prophylaxis, diagnostic facilities and effective treatment is often limited. In a Cape Town study [13], almost two-thirds of deaths in the first few weeks of ART were attributable to wasting syndrome, tuberculosis, acute bacterial infections and malignancy, and immune restoration disease was implicated in 24% of deaths. Although death rates rapidly decrease from those prior to commencement of ART, approximately 66% of ART program deaths occur in the first 4 months of therapy. After 4–6 months of ART, mortality rates approach those seen in Western Europe and North America [11••,12,38].

Mortality on established antiretroviral therapy

Mortality during established ART is predominantly due to a continuing but lower frequency of HIV-related events which occur predominantly in those who do not adhere to therapy or who have blunted CD4-count responses [39•] together with infrequent toxicities associated with therapy [40]. Life-threatening drug-related adverse events can occur early (e.g. hypersensitivity reactions to nevirapine or abacavir) or after several months (e.g. lactic acidosis with d4T). In a Cape Town ART program [13], two ARV-related deaths were reported during 488 patient-years of follow-up, due to a nevirapine-related Stevens Johnson syndrome and stavudine-related lactic acidosis.

Access to less toxic NRTIs would provide additional options to clinicians and patients, but these agents are more expensive or have limited availability in many resource-limited settings [41,42].

Strategies to reduce early antiretroviral therapy mortality

A number of potential contributors to early mortality exist and these are summarized in Table 3.

Guidelines for the initiation of antiretroviral therapy

Early HIV mortality is high in resource-poor settings because of increased exposure to parasitic infections such as malaria, increased exposure to tuberculosis and gastrointestinal parasitic infections as a result of poor water supply and sanitation [43]. In advanced HIV infection, an AIDS diagnosis in resource-poor settings is associated with markedly lower survival than in industrialized settings [44–51]. The risk–benefit analysis of ART compared with no ART would justify early initiation of ART in resource-poor settings in which there is an increased exposure of immune-compromised individuals to pathogenic organisms [43].

Increased access to voluntary counseling and testing

In the USA, where 1–1.2 million are HIV-infected, approximately 25% are unaware of their HIV serostatus and are unable to access the benefits of ARV therapy [26,27]. In contrast, in sub-Saharan Africa, where 24.5 million are HIV-infected, 90% are unaware of their HIV serostatus and therefore do not seek ARV treatment [52]. Currently, individuals in sub-Saharan Africa access therapy after development of serious opportunistic infections rather than because of an awareness of HIV infection. Earlier access to ARV therapy will only be possible if a larger proportion of the HIV-infected population is informed of their status as a result of Voluntary Counseling and Testing (VCT). In order to increase uptake of VCT, Botswana has initiated routine HIV testing in all healthcare facilities (opt-out strategy) [53] and South Africa has introduced provider-initiated HIV testing in the 2007–2011 strategic plan for HIV and AIDS.

Increased CD4 testing

Either a CD4 cell count of less than 200 cells/μl or the presence of an opportunistic illness can define an AIDS diagnosis in the expanded Centers for Disease Control (CDC) definition of AIDS [54]. The prognosis of a CD4 cell count of 200 cells/μl and AIDS is very different, however. The median survival of an individual in South

Table 3 Possible interventions to reduce mortality before antiretroviral therapy initiation and in early and established antiretroviral therapy

Pre-ART	Early ART	Established ART
ART initiation guidelines	Early ART initiation	Community access to free ART
Increased voluntary counseling and testing	Access to free ART	Adherence support
Increased access to CD4 testing	Prophylaxis of opportunistic disease	Active case follow-up
Minimize health-system delays	Management protocols for immune restoration disease	Optimized low-toxicity drug regimens

ART, antiretroviral therapy.

Africa with an AIDS diagnosis is 6–12 months, whereas the median survival from a CD4 cell count of 200 cells/μl is 18–24 months [55]. If patients in sub-Saharan Africa could access therapy when their CD4 count had declined to a count of 200 cells/μl, rather than when an AIDS condition had been diagnosed, then mortality could be markedly reduced both before ART and during early ART periods. Wider access to CD4-cell monitoring is therefore needed, particularly for those presenting to healthcare facilities with nonAIDS conditions, such as at VCT, family planning and maternity services, sexually transmitted infection clinics and tuberculosis diagnostic and treatment facilities. Knowledge of the CD4 cell count also promotes the initiation of prophylaxis against opportunistic disease, with subsequent improved survival [35,56].

Reducing health-system delay

In sub-Saharan Africa, 90% of HIV-infected individuals are unaware of their HIV serostatus [52] and many enter medical care with symptoms and signs of advanced HIV infection or with a diagnosis of an opportunistic infection. A diagnosis of AIDS, however, already indicates that the threshold for ART eligibility has already been crossed and the median survival of such patients is only 6–12 months [55]. Therefore, each subsequent month's delay in accessing ART after presenting to the health system with AIDS is associated with a 4–8% mortality.

Delayed referral may be due to 'therapeutic nihilism' of health workers who have worked in healthcare settings in which extraordinarily high AIDS mortality has been accepted as normal [57]. ART is frequently only available at a limited number of sites [58] and referrals across fragmented health systems also may result in further delays [36]. Facilities dispensing ARVs have also been subject to rapid expansion and are frequently logistically constrained, resulting in long waiting lists of several months to receive ART [32]. All of the delays due to referral, health system and waiting list contribute to both pre-ART mortality and late initiation of ART. To date, published studies of the effectiveness of ART in resource-poor settings have concentrated on morbidity and mortality after the initiation of ART and the high mortality associated with delay in accessing ART has remained largely unrecognized [13].

Optimizing antiretroviral therapy programs

In-program mortality may be seriously underestimated in ART treatment programs which have not allocated resources for active follow-up of patients, as high loss to follow-up rates may hide true on-treatment mortality [11••]. Reporting systems incorporating active follow-up of patients allow more accurate measurement of program performance together with the early identification of those for whom the program is failing.

In the developing world, many HIV-infected patients are poor and are subject to other survival needs which compete with the need for accessing medical care. An across-program analysis [11••] of heterogeneous ART programs in the southern hemisphere has identified that the requirement to make a financial copayment for drugs, medical services or investigations was associated with a two-fold increase in ART mortality. The provision of free ART within medical programs is therefore likely to reduce on-treatment mortality. There may be other costs for accessing care, however, particularly in rural areas in which costs of transport are high [11••]. Innovative models of successful community delivery of ART have been developed in the poorest of settings [59,60].

Adherence to treatment has been a neglected area of many medical interventions. Adherence to treatment, however, has been a strong and continuing focus of the public-health approach to expanded access to ART [7,61]. Despite initial skepticism that necessary levels of adherence could be achieved in resource-poor populations [62], there have been many studies [63–66] from ART programs documenting high levels of adherence and viral suppression rates matching those achieved in developed-world settings. A relative shortage of nurses and doctors has encouraged the innovative use of non-medically qualified community members for treatment support within programs. In rural Haiti, community 'accompagnateurs' have been used to deliver ART [59]; in urban South Africa, relatives and friends have been used as 'treatment buddies' [66] and HIV-infected individuals trained as 'therapeutic counselors' [60,65]. Use of these and other innovative adherence strategies will continue to be necessary to address the continuing challenge of maintaining high levels of adherence, particularly as programs continue to scale up.

Increased access to ART has followed the marked reduction in ARV drug prices. While program effectiveness is increased by improved adherence, program costs are still driven by the price of first-line regimens [42,67]. Consequently, many programs utilize ARVs because of their cheapness with adverse toxicity profiles which may result in on-treatment deaths [68–70]. A need for access to cheaper and less toxic drugs therefore continues.

Patients currently access ART programs in sub-Saharan Africa when CD4 cell counts are low [12,65,66] and coinfections are frequent [71]. Delays due to diagnosis and treatment of coinfections result in postponed initiation of ART and contribute to very high mortality rates [72,73]. The very high mortality of coinfected patients may be reduced by earlier initiation of ART [66] but such a policy is associated with increased rates of immune restoration disease (IRD) [74]. IRD may be

mild, resulting in local discomfort from enlarged peripheral tuberculous nodes, or may be life-threatening with central nervous system involvement of tuberculosis or cryptococcal infections [75,76]. The high frequency of IRD together with its potentially lethal complications reinforce the need for standardized management protocols for both treatment and prevention of severe IRD.

Conclusion

The HIV epidemic has spread by exploiting the frailties and weaknesses of human social behavior; similarly, high morbidity and mortality have exposed weaknesses and inequalities of healthcare systems. The scale of the HIV epidemic in resource-poor settings has necessitated a public-health approach to the expanded access of ART and a critical analysis of health systems. While virologic and immunologic responses achieved by this approach are similar to those reported in industrialized settings, mortality early after starting ART is very high. This mortality is primarily due to a high pre-ART morbidity compounded by advanced immune suppression at the time of accessing ART. IRD and drug toxicities may add to this already high mortality. Early ART program deaths may be reduced by implementation of measures which encourage earlier access to care for HIV-infected individuals in resource-poor settings.

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Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa

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Two-thirds of the world's HIV-infected people live in sub-Saharan Africa, and more than 1.5 million of them die annually. As access to antiretroviral treatment has expanded within the region; early pessimism concerning the delivery of antiretroviral treatment using a large-scale public health approach has, at least in the short term, proved to be broadly unfounded. Immunological and virological responses to ART are similar to responses in patients treated in high-income countries. Despite this, however, early mortality rates in sub-Saharan Africa are very high; between 8 and 26% of patients die in the first year of antiretroviral treatment, with most deaths occurring in the first few months. Patients typically access antiretroviral treatment with advanced symptomatic disease, and mortality is strongly associated with baseline CD4 cell count less than 50 cells/μl and WHO stage 4 disease (AIDS). Although data are limited, leading causes of death appear to be tuberculosis, acute sepsis, cryptococcal meningitis, malignancy and wasting syndrome. Mortality rates are likely to depend not only on the care delivered by antiretroviral treatment programmes, but more fundamentally on how advanced disease is at programme enrolment and the quality of preceding healthcare. In addition to improving delivery of antiretroviral treatment and providing it free of charge to the patient, strategies to reduce mortality must include earlier diagnosis of HIV infection, strengthening of longitudinal HIV care and timely initiation of antiretroviral treatment. Health systems delays in antiretroviral treatment initiation must be minimized, especially in patients who present with advanced immunodeficiency.

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Introduction

Development of highly active antiretroviral treatment (ART) in the mid-1990s revolutionized the care of HIV-infected patients and led to marked reductions in HIV-associated morbidity and mortality in many industrialized countries [1–3]. Over 10 years later, access to ART remains limited in sub-Saharan Africa and other resource-constrained settings where the need is greatest. Although only 10% of the world's population live in sub-Saharan Africa, the region is nevertheless home to around two-thirds of the world's HIV-infected people [4]. In 2007, an estimated 22.5 million adults and children in the region were living with HIV/AIDS and 1.6 million died, representing 76% of global AIDS deaths [4]. Great progress has been made in providing access to ART in sub-Saharan Africa; by April 2007, approximately 1.3 million people were receiving ART – some 28% of the 4.8 million people estimated to be in need [5]. However, in addition to much needed HIV prevention measures, ongoing expansion of capacity to effectively deliver ART long-term to large numbers of people is urgently required.

Early pessimism concerning effective delivery of ART on a large scale using a simplified public health approach has largely proven unfounded, at least in the short term. For example, ART access has been rapidly expanded on a massive scale in Lusaka, Zambia, in Abidjan, Cote D'Ivoire, and on a country-wide scale in Malawi with good early clinical outcomes [6–8]. High levels of treatment compliance and virological suppression have been achieved in both hospital-based and community-based programmes [9–11]. Meta-analyses of data from treatment cohorts have found that the efficacy of ART, as reflected by rates of viral load suppression and CD4 cell count recovery, is similar among patients treated in high-income and resource-limited settings [12,13]. ART has been demonstrated to be a cost-effective intervention in resource-poor settings [14–16]. Furthermore, ART was estimated to have averted 250 000–350 000 deaths in low and middle-income countries in 2005 alone [17].

Despite these positive findings, data from the region suggest that early mortality rates among adults in ART programmes are high [12], contributing to substantial losses in overall patient retention [18]. The ART–lower income country (ART-LINC) collaboration compared outcomes from 18 ART programmes in lower income settings (predominantly in Africa) with those in 12 HIV cohort studies from Europe and North America [12]. This analysis found that early mortality following initiation of ART was several-fold higher among patients in resource-limited settings compared to that of patients treated in high-income settings, even after adjusting for baseline immunodeficiency [12]. High mortality rates in individual cohorts in sub-Saharan Africa have also been reported on. To gain a greater understanding of this problem, we review the mortality rates, temporal distribution, risk factors and causes of death among adult patients accessing ART programmes in sub-Saharan Africa and consider possible strategies to address this.

Search strategy and selection criteria

We searched English-language publications in MEDLINE, using the terms 'antiretroviral*', 'Highly Active Anti-Retroviral Therapy (HAART)', 'ART', 'Africa', 'mortality', 'death'. Searches were completed up to December 2007 to identify published reports from observational ART cohorts in sub-Saharan Africa; those reporting on survival proportions over time and those describing the spectrum of causes of death were selected. Additional articles were identified from references in published papers and abstracts from major international AIDS conferences in the preceding 12 months. For cohorts with multiple presentations of mortality data from different time periods, the most recent report was used. Data from controlled clinical trials were not included in the assessment of mortality rates as these data are unlikely to be generalizable to the scale-up of ART services in the general population. Summary estimates of the hazard ratios for the association of early mortality with CD4 cell count and with advanced WHO stage was conducted using a random-effects model [19].

Rates and temporal distribution of mortality

Data from 18 published cohort studies containing 39 536 patients treated in countries in west, east and southern Africa are summarized in Table 1 [20]. Patients were mainly receiving public sector treatment. Twelve of the cohorts were based in urban settings and six were rural. The median baseline CD4 cell count in these studies ranged between 43 and 147 cells/μl, and the median duration of follow-up ranged between 3 and 46 months. The vast majority of these patients were ART-naive, and most initiated triple-drug therapy that incorporated two nucleoside analogues and a nonnucleoside reverse transcriptase inhibitor.

Using product-limit methods, the 12-month survival proportion in patients who were not lost to follow-up ranged between 0.74 and 0.92, with the greatest burden of mortality occurring during the initial months of ART (Table 1). Heterogeneity in outcomes between programmes may reflect differences in both baseline patient characteristics and programme characteristics [21]. Survival proportions were reported at both 12 and 24 months of follow-up in nine studies and these

Table 1. Mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa.

Study	Year	Country	*n*	Setting	Median (IQR) CD4 cell count (cells/μl)	Median follow-up (months)	Survival proportion: On-treatment (months) 12	On-treatment (months) 24	At 12 months of ART <50[a]	At 12 months of ART >50[a]	Temporal distribution of deaths	Losses to follow-up
Weidle *et al.* [26]	2002	Uganda	476	Urban	73 (15–187)	3	0.74	–	0.67	0.82	57% deaths in first 3 months	24%
Djomand *et al.* [31]	2003	Cote D'Ivoire	480	Urban	[37% <50]	6	0.84	–	–	–	–	Not reported
Seyler *et al.* [32]	2003	Cote D'Ivoire	101	Urban	135 (55–221)	17	–	–	0.80	0.96	50% deaths in first 3.3 months	Not reported
Coetzee *et al.* [10]	2004	S. Africa	287	Urban	43 (13–94)	13.9	0.86	0.86	0.82	0.91	71% deaths in first 3 months	2%
Laurent *et al.* [41]	2005	Senegal	176	Urban	144 (58–224)	30	–	0.84	–	–	Median time to death 9 months	Not reported
Wester *et al.* [11]	2005	Botswana	153	Urban	96 (33–165)	12	0.85	–	0.76	0.90	Majority of deaths in first 6 months	8%[c]
Lawn *et al.* [23]	2006	S. Africa	927	Urban	100 (47–160)	7	0.91	0.90	0.85	0.94	87% between enrolment and first 4 months ART	2%
Ferradini *et al.* [25]	2006	Malawi	1308	Rural	112 (59–176)	8.3	0.81	0.72	–	–	77% deaths in first 6 months	5%[c]
Etard *et al.* [24]	2006	Senegal	404	Urban	128 (54–127)	46	0.88	0.83	0.80	0.90	50% deaths in first 12 months	2% at 1 year
Stringer *et al.* [6]	2006	Zambia	16198	Urban	147 (69–268)	7	0.82	–	0.80	–	71% within first 3 months	Not reported
Zachariah *et al.* [37]	2006	Malawi	1507	Rural	123 (58–206)	10[b]	0.87	–	0.77	0.92	61% in first 3 months; 79% in first 6 months	3%
Makombe *et al.* [20]	2007	Malawi	4580	Rural	–	12	0.87	–	–	–	>90% in first 6 months	11% at 1 year
Bajunirwe *et al.* [39]	2007	Uganda	398	Rural	–	–	0.76	0.71	–	–	–	Not reported
De Iaco *et al.* [34]	2007	Burkina Faso	315	Urban	97	18	–	–	0.69	0.83	Mortality rate 2.3-fold higher in first 6 months ART	Not reported
Johannessen *et al.* [38]	2007	Tanzania	336	Rural	–	12	0.74	0.68	–	–	60% deaths in first 3 months	11%
Kambugu *et al.* [52]	2007	Uganda	559	Urban	104	12	0.86	–	–	–	73% in first 6 months	Not reported
Moore *et al.* [40]	2007	Uganda	1120	Rural	127	24	0.92	0.91	–	–	68% in first 6 months	Not reported
Toure *et al.* [8]	2008	Cote D'Ivoire	10211	Urban	123 (47–207)	7.7	–	–	0.76	>0.86	75% in first 4.6 months	19%[c,d]

ART, antiretroviral therapy; IQR, interquartile range.
[a]CD4 cell count (cells/μl).
[b]Mean (SD).
[c]At 12 months. Kaplan–Meier estimate.
[d]Total losses at 12 months.

showed that mortality accruing in the second year of ART was much less than that in the first (Table 1).

Longitudinal changes in mortality rates were calculated in some studies [21–24]. In a South African cohort, the rate in the first 4 months of ART was 19.1 deaths/100 person-years (100PYs), decreasing to 2.9 deaths/100PYs beyond 4 months and 1.3 deaths/100PYs beyond 1 year [23]. A similar pattern of mortality was observed in another South African cohort [10], and in both studies, rates of viral load suppression of less than 400 copies/ml were high (>93% and >89%) [10,23]. In other studies, however, substantial mortality accrued between 12 and 24 months of follow-up [24,25]. Mortality rates in a cohort in Senegal, for example, were 12.5, 6.6 and 4.5 deaths/100PYs in the first, second and third years of treatment, respectively [24]. The higher mortality rates beyond the first year of ART in these studies might be explained by lower rates of virological suppression and associated poor immunological recovery, for example [24,26].

In all 18 cohorts (Table 1), the mortality proportions at 12 months of follow-up (range 8–26%) exceeded the ART–LINC summary mortality estimate of 6.4% [95% confidence interval (CI), 5.1–7.7] [12]. The ART–LINC estimate was derived from 4810 patients receiving ART between 2001 and 2004 in resource-limited settings and included 3449 patients in sub-Saharan Africa. Since then, there has been a more than 10-fold increase in the number of patients receiving ART in the region whose outcomes have been published. The current literature included in this review suggests that further updated collaborative analyses might show a substantially higher early-mortality rate than initially derived from the original ART–LINC cohorts.

Reliability of mortality data

There may be a tendency for mortality rates in the published literature to be lower than those observed in most large-scale ART programmes that have not published their data because of selective reporting from well run ART programmes in urban settings with more intensive service delivery and fewer resource constraints. The quality of mortality data is unclear in some reports; mortality is likely to be underestimated to a degree by most cohorts due to misclassification of unascertained patient deaths as losses to follow-up. The greater the loss to follow-up rate the greater the potential for misclassifications. Rates of loss to follow-up differed greatly between studies, ranging between 2 and 24% (Table 1), and unrecorded mortality among these patients may only be detected by intensive active follow-up [12,27–29]. The efficacy of active follow-up may vary considerably between cohorts and details concerning the intensity of patient tracing are infrequently reported.

Analysis of the characteristics of losses to follow-up may provide insights into the reliability of programme mortality data. ART cohorts in South African and Cote D'Ivoire with good ascertainment of outcomes found that, whereas most deaths occurred in the first months of ART in patients with the lowest baseline CD4 cell counts, losses to follow-up were evenly distributed over time and were not associated with CD4 cell counts [8,23]. Thus, if a programme reports a high loss to follow-up rate in the first months of ART among patients with the lowest baseline CD4 cell counts, this may be suggestive of high rates of unascertained deaths in this period.

In-programme mortality prior to starting anti-retroviral therapy

Very high mortality rates recorded during the initial months of ART may reflect a high mortality rate among individuals who are eligible for ART but have yet to start treatment. This includes individuals enrolled in care who are awaiting treatment as well as those elsewhere in the healthcare system awaiting referral to HIV treatment services.

Two cohorts in South Africa have reported on deaths occurring in the interval between enrolment of patients into the ART programme and initiation of treatment [22,23,30]. In the Cape Town study, this interval of approximately 30 days permitted thorough investigation and treatment of coinfections and preparation of patients for ART, the mortality rate in this interval was very high (approximately 30 deaths/100PYs). Deaths occurring in this short period accounted for 67% of deaths within the first 3 months from programme enrolment [22]. Similarly, in the Free State cohort, 87% of deaths occurred among patients prior to ART initiation [30]. Thus, it is likely that even short delays in ART initiation are associated with considerable pretreatment mortality risk. Delays in patient referral, waiting lists for ART initiation and time taken to prepare patients to start life-long treatment are likely to contribute to overall mortality risk.

Such delays and associated mortality are not typically reported by cohorts and so the optimum period for preparation for ART is therefore unclear. How to balance the need for thorough preparation of patients for life-long therapy with the high risk of death among individuals waiting to start therapy requires urgent research attention. Clearly, flexibility in timing is needed as many patients with advanced immunodeficiency need therapy urgently.

Risk factors for mortality

Of the cohorts summarized in Table 1, low baseline CD4 cell count was a strong risk factor for early mortality in all those with available data. The summary hazard ratio for the association between CD4 count of less than 50 cells/μl (versus CD4 >50 cells/μl) was 2.5 (95% CI, 1.9–3.2) in studies with data presented in this format [10,11,22,25,26,31,32]. A graded association with CD4 cell count was reported in some studies [6,12,23,33] (Fig. 1a) but baseline viral load was not found to be an independent risk factor in any of them.

Symptomatic disease (WHO stages 3 and 4) was associated with mortality in some [6,8,12,22,34] but not all [11,22] studies (Fig. 1b), possibly reflecting differences in the accuracy of clinical staging or homogeneity of cohorts with respect to this variable. WHO stage 4 disease, however, was found to be a strong predictor of mortality in all studies reporting on this [6,22,23,25,31,35–39]. In three studies comparing patients with WHO stage 4 disease at baseline with those with WHO stages 1–3, WHO stage 4 was associated with more than a doubling in the hazard of death (summary hazard ratio, 2.2; 95% CI, 1.5–3.2) [10,21,31].

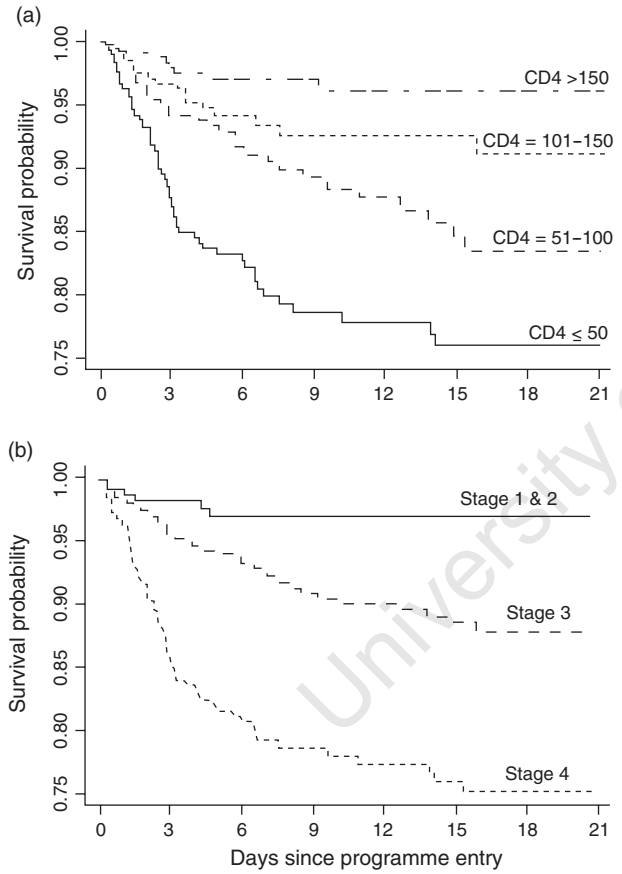

Fig. 1. Kaplan–Meier plot showing survival proportion over 21 months among patients ($n = 1235$) from the time of entering a community-based antiretroviral treatment programme in South Africa. The period covered includes the interval between programme enrolment and ART initiation – a median of 33 days. The survival proportions are shown according to baseline CD4 cell count and baseline WHO clinical stage. The graphs show the strong unadjusted association between CD4 cell count, WHO clinical stage and mortality risk in the first year of ART and the low mortality risk in the second year. ART, antiretroviral treatment. Adapted from [23].

Low body mass index [6,8,24,25,35,37,38,40] and anaemia [6,8,25,38,40,41] were independently associated with mortality in some studies. Anaemia may be associated with a variety of conditions such as extra-pulmonary tuberculosis (TB), gastrointestinal Kaposi's sarcoma and severe malnutrition or may reflect the direct effects of advanced HIV on haematopoiesis. Male sex was associated with poorer survival in six studies [6,8,10,23, 25,39]; the reasons for this are unknown, but could relate to differences in health-seeking behaviour or poorer treatment adherence among men [42].

In contrast to data from high-income settings [2], increasing age was associated with higher mortality risk in only one cohort [8]. The lack of an association may reflect the younger and narrower age distribution of patients receiving ART in Africa. Alternatively, this may simply reflect the relatively short duration of follow-up in these studies since immunological recovery during the first 4 months of treatment is not age-dependent whereas long-term recovery is [43–45].

Patients with TB at enrolment to ART programmes have a high mortality risk [46,47], and yet, paradoxically, TB and TB disease activity have not been found to be independent risk factors for mortality during ART [6,37,48]. Many deaths, however, may occur before ART is initiated [48,49], and also much of the true burden of disease may remain unascertained [50]. In contrast to TB, detection of cryptococcal antigen in serum was an independent predictor of early mortality in a rural cohort in Uganda [51].

Although the type of ART regimen and healthcare facility (primary versus secondary) have not been found to be associated with mortality [8,12,24–26], programmes in which patients were required to pay for medication were associated with a four-fold greater mortality risk [12]. This concurs with the findings of a meta-analysis of treatment outcomes in resource-poor settings; due to limitations in finances, patients who had to pay part or all the cost of therapy had an approximately 30% lower probability of having an undetectable viral load at 6 and 12 months compared with patients whose medication was supplied free of charge [13]. In keeping with this, ART adherence has been found to be predictive of mortality [40,42].

Risk factors for mortality may alter during the course of ART. Whereas mortality during the first 4 months of treatment in one cohort was associated with patient characteristics at baseline, mortality beyond this time point was only associated with the updated absolute CD4 cell count at 4 months and with failure of viral load suppression [23]. These data suggest that the key long-term determinant of mortality is the response to ART as also suggested by more recent findings from Abidjan [35].

Causes of death

Five observational cohort studies from a range of countries in sub-Saharan Africa report on the spectrum of causes of early deaths [22,24,37,40,52]. The most important causes of death reported include TB, acute sepsis, cryptococcal meningitis, malignancies (especially Kaposi's sarcoma) and chronic diarrhoea or wasting syndrome.

Diagnostic facilities are limited in many settings, especially for rural cohorts, and no identifiable cause could be identified in a proportion of deaths. No systematic post-mortem studies of deaths during ART have been reported and the overall data are therefore limited. Deaths in the first few weeks of ART are likely to be caused by conditions that are either preexisting at programme enrolment or new conditions arising in the context of persisting immunodeficiency. In one study, causes of death during the first 4 months of ART were found to reflect those occurring just prior to ART with the addition of deaths due to immune reconstitution disease associated with cryptococcal meningitis and TB [22].

TB was among the two leading causes of death in four of the five cohorts, accounting for up to 21% of deaths [22,24,40,52]. However, TB is likely to be an under-reported cause of death. Post-mortem studies in the pre-ART period have found that up to 54% of untreated patients dying from AIDS in Africa have evidence of occult disseminated TB [53,54], and that this may be a common underlying cause of 'slim disease' [55]. Although wasting syndrome (defined as wasting with unexplained fever or chronic diarrhoea or both) was specifically reported as an important cause of death among in only one cohort [22], this may have been more common as 'chronic diarrhoea' was also reported as a common cause [37], and wasting was a common risk factor for mortality in many cohorts (see earlier).

The contribution of TB immune reconstitution disease to mortality has yet to emerge fully [56]. Although most cases appear to be self-limiting, a minority are severe, and deaths have been reported from cohorts in South Africa and Thailand [57,58]. However, background mortality rates were also very high among TB patients who did not develop this complication, and neither of the studies found immune reconstitution disease to be associated with a significantly excess mortality risk. Larger prospective studies are needed to clarify this issue.

Cryptococcal meningitis was among the two leading causes of mortality in four of the five cohorts [22,24,40,52], accounting for up to 20% of deaths. Many of the deaths from cryptococcal disease in a South African cohort were attributed to immune reconstitution disease [59] and this was a more common cause of death than TB immune reconstitution disease [22]. Many patients developing this complication have previously been treated for cryptococcal meningitis with fluconazole monotherapy [22,59,60]. Although this is the standard of care throughout much of Africa, fluconazole is a fungistatic drug with far less efficacy than amphotericin in clearing the organism from cerebrospinal fluid (CSF) and especially so in the context of fluconazole resistance [61]. Persistence of cryptococci or cryptococcal antigen in the CSF is likely to predispose patients to immune reconstitution disease, which has a high mortality risk [59,60,62,63].

Kaposi's sarcoma and other malignancies were among the three leading causes of death in three of the five cohorts [22,37,52], accounting for up to 14% of deaths. Acute sepsis was another important cause identified in some cohorts despite widespread use of trimethoprim-sulphamethoxazole (cotrimoxazole) [22,40]. Acute respiratory disease, acute gastroenteritis or systemic sepsis with no identifiable focus were the most common forms of sepsis and collectively sepsis accounted for up to 19% of deaths. *Pneumocystis jiroveci* pneumonia was reported in up to 9% of deaths in two Ugandan studies [40,52] but the basis for these diagnoses is not clear.

Microbiological causes of death from acute bacterial infections have not been reported. In studies of acute sepsis during cotrimoxazole prophylaxis in Abidjan, Cote D'Ivoire [32,64–66], the predominant pathogens were nontyphoidal salmonella, *Escherichia coli*, *Shigella* spp. and *Streptococcus pneumoniae*. Although high rates of cotrimoxazole resistance among nontyphoidal salmonellae and pneumococcal isolates have been reported in Uganda and Malawi [67,68], prophylaxis with this drug is still effective in reducing mortality in both countries [68,69].

The most widely used regimens in Africa include stavudine (d4T) and nevirapine, which have the potential for serious toxicities that include hepatotoxicity, Stevens–Johnson syndrome and lactic acidosis [70,71]. However, drug toxicity does not appear to be a major cause of early mortality. Among 226 deaths reported by four cohorts, seven (3.1%) were attributed to ART toxicity [10,22,24,52]. These were comparatively well resourced

programmes with access to biochemical laboratory monitoring; mortality rates may be higher where monitoring is not possible. Analysis of the temporal distribution of drug substitutions due to toxicity shows that nevirapine and zidovudine toxicity occur during the first few months of ART, whereas d4T-associated lactic acidosis occurs cumulatively from around 6 months onwards [72,73]. Thus, the contribution of drug toxicity to overall mortality may change over time and may proportionately increase as the risk of opportunistic infections diminishes after the first 6 months of ART.

Deaths associated with nevirapine-induced hepatotoxicity have been reported from South Africa when used concurrently with rifampicin [70], but more data from large cohorts are needed to quantify the risk during concurrent TB treatment. In contrast, efavirenz is very well tolerated [65], and simultaneous use of rifampicin and efavirenz in cohorts in South Africa has not been associated with mortality [48,74].

Strategies to reduce mortality

Early mortality rates are strongly associated with the degree of immunodeficiency in patients at the time they enrol into ART programmes. Strategies to reduce mortality must therefore focus not only on delivery of care within ART programmes but more fundamentally they must promote early HIV diagnosis and improved pre-ART HIV care.

(1) Early HIV diagnosis and longitudinal HIV care pre-ART
  (a) Promote HIV testing and early diagnosis.
  (b) Strengthen long-term HIV care services for patients prior to ART eligibility.
  (c) Optimize prevention, screening and treatment of opportunistic infections.
  (d) Provide longitudinal clinical and CD4 cell count monitoring to facilitate timely initiation of ART.
  (e) Minimize health system delays in ART initiation.

(2) Delivery of ART
  (a) Provide ART without charge to the patient.
  (b) Use updated WHO guidelines for ART eligibility.
  (c) Develop locally effective ART adherence strategies.
  (d) Use ART regimens with lower toxicity.
  (e) Opportunistic infections
    (i) Cotrimoxazole prophylaxis during ART.
    (ii) Optimize screening for TB at ART programme entry.
    (iii) Optimize diagnosis and management of TB immune reconstitution disease.
    (iv) Prevent nosocomial transmission of TB.
    (v) Consider concurrent isoniazid preventive therapy (IPT) during ART to reduce long-term TB incidence (trial data awaited).
    (vi) Optimise prevention, diagnosis and treatment of cryptococcal meningitis and immune reconstitution disease.
  (f) Use of laboratory monitoring
    (i) Laboratory monitoring for drug toxicity.
    (ii) Define appropriate strategies for monitoring response to ART.

Early HIV diagnosis and longitudinal HIV care

Patients entering ART programmes in sub-Saharan Africa have typically had their HIV diagnosis made following presentation to the health services with advanced symptomatic disease. Such patients have a high mortality risk in the period leading up to ART as well as during early ART (Fig. 2). Provision of accessible and user-friendly services for serial voluntary counselling and testing (VCT) and CD4 cell count estimation is vital to promote early HIV diagnosis and assessment of ART eligibility. Early diagnosis and initiation of appropriate longitudinal care would probably be associated with much lower mortality risk in the period leading up to ART as well as during the initial months of ART (Fig. 2).

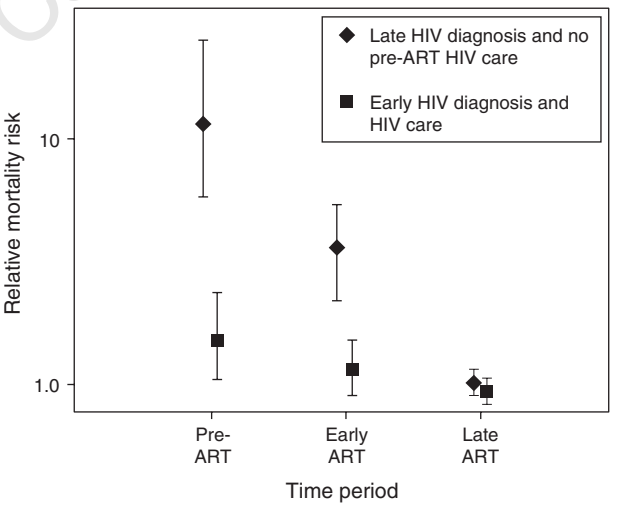

Fig. 2. Hypothetical graph showing relative mortality risk (95% confidence interval) among patients accessing antiretroviral treatment with late HIV diagnoses and advanced symptomatic disease (diamonds) compared to patients whose HIV was diagnosed early and who received appropriate longitudinal HIV care and timely referral for antiretroviral treatment (squares). Mortality risk is shown broken down into 3 periods: pre-ART (the interval between enrolment in the ART programme and the time ART is started), early ART (first 4 months of ART) and late ART (beyond 4 months of ART). ART, antiretroviral treatment. Data on patients with late diagnoses are based on [23] and data from patients with early diagnoses and care are hypothetical, being based approximately on data from [23,33,82].

Similar to the management of many chronic diseases in Africa, longitudinal care from the time of HIV diagnosis until requirement for ART is often poor. Thus, even those patients who are diagnosed with relatively early HIV infection are often lost to medical care only to later re-enter medical services with advanced disease. Longitudinal care must be strengthened and should include screening for and treatment of opportunistic infections, initiation of cotrimoxazole prophylaxis, isoniazid preventive therapy (IPT), reproductive healthcare, and serial CD4 cell count assessment until eligibility for ART is reached. This will require strengthening of HIV care services and more widespread availability of CD4 cell count measurement.

Prevention, screening and management of opportunistic infections are needed throughout the HIV care pathway. Cotrimoxazole prophylaxis should be initiated after HIV diagnosis and continued during ART [75], as this is associated with additional gains in life expectancy [16,76]. Isoniazid preventive therapy (IPT) is an underused intervention in Africa [77]. Key obstacles include the difficulty of excluding active TB in patients with moderate or advanced immunodeficiency. IPT would therefore be more easily used among patients with less advanced disease, and yet, adherence to treatment among such patients and the infrastructure to efficiently deliver and monitor IPT remain a challenge.

Delivery of anti-retroviral therapy

National ART programme criteria for ART eligibility require ongoing re-evaluation. Initial WHO guidelines (2002) for resource-poor settings recommended treatment only for those with stage 4 disease or a CD4 cell count of less than 200 cells/μl [78]. These were modified in 2003 [79] and 2006 [80], bringing them closer to guidelines for high-income settings. However, some national programmes had earlier retained more restrictive eligibility criteria. For example, South Africa's national programme has retained the 2002 WHO recommendations despite the fact that under these guidelines much of HIV-associated morbidity and mortality occurs prior to eligibility for ART [81,82]. Studies on when to initiate ART in resource-limited settings are needed. There are currently two trials that aim to address this issue in Cote D'Ivoire and Haiti (http://clinicaltrials.gov/ct2/show/NCT00120510?term=NCT00120510&rank=1, http://clinicaltrials.gov/ct2/show/NCT00495651?term=Temprano&rank=1).

As soon as patients are identified as being eligible for ART, prompt referral for ART should be made. Where possible, waiting lists for ART should be minimized and those with highest risk might be prioritized [22,24]. Supply of medication free of charge to the patient is a key issue [12,13]. While some international agencies advocate the privatization of health services and private financing of health services through user fees, this is very unrealistic for most patients living in resource-limited settings who require life-long ART.

The use of regimens in Africa with higher toxicity than those used in the West is due to their low cost and the availability of fixed dose combination formulations. Although provision of treatment to the millions of people living with HIV/AIDS who do not yet have treatment access should be the priority, implementation of less toxic regimens is nevertheless desirable.

Although laboratory monitoring and use of point-of-care lactic acid meters may help detect and manage drug adverse effects, no data yet exist to indicate that provision of such monitoring reduces mortality risk. This issue is being studied in the Development of AntiRetroviral Therapy in Africa trial (DART, http://www.ctu.mrc.ac.uk/dart/summary.asp). Braitstein *et al.* [12] found no impact of viral load monitoring on mortality in the first year of ART. However, any benefits are only likely to occur during long-term treatment rather than during the first year.

Effective strategies to screen for active TB at entry to ART programmes need to be developed, including detection of the high prevalence of both clinical and subclinical disease [46,47,83,84]. Prompt initiation of TB treatment may reduce patient mortality and reduce risks of nosocomial TB transmission. The optimal timing for initiation of ART among TB patients remains unknown and randomized controlled trials of early versus delayed initiation of ART is currently underway [85]. Similar to data from the UK [86,87], observational data from South Africa strongly suggest that expedited initiation of treatment is needed among those with baseline CD4 cell count of less than 100 cells/μl in this setting in view of their exceptionally high mortality rate while awaiting ART [88,89].

Randomized controlled trials are needed to define both the optimal management of moderate and severe TB immune reconstitution disease [56] and to assess the efficacy of concurrent isoniazid prophylaxis in reducing the high rates of TB that persist during ART [46,90]. In addition, the utility of pre-ART screening for and management of asymptomatic cryptococcal antigenaemia has yet to be defined [51]. Provision of a better standard of care for cryptococcal meningitis using amphotericin might be considered in settings with adequate facilities to administer this drug safely. Guidelines for the prevention, diagnosis and management of cryptococcal immune reconstitution disease are also needed.

Despite early reports that treatment adherence does not pose a major barrier to treatment success in sub-Saharan Africa [91], more recent research suggests that overall adherence rates and retention on ART in sub-Saharan

Africa are quite variable and often poor [18,92]. Adherence is predictive of mortality [42] and development of locally appropriate strategies to promote adherence are central to the success of ART programmes. Research is also needed to determine which cadre of health-care professional is needed to deliver ART effectively with good outcomes.

Conclusion

High early mortality within ART programmes in sub-Saharan Africa has emerged as a key challenge. Between 8 and 26% of patients die in the first year of ART and key issues surrounding this problem are summarized as follows:

(1) Although virological and immunological responses to ART are similar in patients living in high-income and resource-limited countries, early mortality rates are much higher.
(2) Most deaths occur in the first few months of ART.
(3) A high loss to follow-up rate may conceal true mortality rates.
(4) High mortality rates during early ART also reflect high mortality rates in the period preceding ART.
(5) Key risk factors for early mortality include low CD4 cell count, advanced clinical stage of disease, and the need for patients to pay for treatment.
(6) Early deaths largely reflect the spectrum of causes of death prior to ART initiation plus immune reconstitution disease.
(7) Common causes of death are TB, acute sepsis, cryptococcal meningitis malignancies and wasting syndrome.
(8) Drug adverse effects are a relatively minor cause of early mortality.
(9) Strategies to reduce early mortality include promotion of early HIV diagnosis, strengthening of the patient care pathway pre-ART, timely initiation of ART, provision of ART free of charge to the patient, adherence support, and optimal prevention, screening and management of opportunistic infections.

Early death rates threaten the credibility of ART delivery among communities accessing such therapy and among health workers who are responsible for providing this care. Although many factors are likely to contribute to this mortality, an over-riding issue is that patients typically present for ART once they have developed advanced symptomatic disease. Much may be done within ART services to potentially reduce this mortality by providing medication free of charge, implementing effective screening, treatment and prevention of opportunistic infections, reinforcing treatment adherence and using regimens with low toxicity. However, despite optimizing ART delivery, a proportion of early deaths among patients with very advanced disease are not likely to be preventable. Thus, a more fundamental issue and the greater challenge is the need for early HIV diagnosis and provision of appropriate longitudinal HIV care prior to ART eligibility.

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Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa

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Objective: To determine the relationship between mortality risk and the CD4 cell response to antiretroviral therapy (ART).

Design: Observational community-based ART cohort in South Africa.

Methods: CD4 cell counts were measured 4 monthly, and deaths were prospectively ascertained. Cumulative person-time accrued within a range of updated CD4 cell count strata (CD4 cell-strata) was calculated and used to derive CD4 cell-stratified mortality rates.

Results: Patients (2423) (median baseline CD4 cell count of 105 cells/μl) were observed for up to 5 years of ART. One hundred and ninety-seven patients died during 3155 person-years of observation. In multivariate analysis, mortality rate ratios associated with 0–49, 50–99, 100–199, 200–299, 300–399, 400–499 and at least 500 cells/μl updated CD4 cell-strata were 11.6, 4.9, 2.6, 1.7, 1.5, 1.4 and 1.0, respectively. Analysis of CD4 cell count recovery permitted calculations of person-time accrued within these CD4 cell-strata. Despite rapid immune recovery, high mortality in the first year of ART was related to the large proportion of person-time accrued within CD4 cell-strata less than 200 cells/μl. Moreover, patients with baseline CD4 cell counts less than 100 cells/μl had much higher cumulative mortality estimates at 1 and 4 years (11.6 and 16.7%) compared with those of patients with baseline counts of at least 100 cells/μl (5.2 and 9.5%) largely because of greater cumulative person-time at CD4 cell counts less than 200 cells/μl.

Conclusion: Updated CD4 cell counts are the variable most strongly associated with mortality risk during ART. High cumulative mortality risk is associated with person-time accrued at low CD4 cell counts. National HIV programmes in resource-limited settings should be designed to minimize the time patients spend with CD4 cell counts less than 200 cells/μl both before and during ART.

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Introduction

The countries of sub-Saharan Africa have been hit hardest by the HIV/AIDS epidemic with an estimated 22.5 million adults and children living with the infection in 2007 [1]. Of these, 1.6 million died, representing 76% of global AIDS deaths that year. Expansion of access to antiretroviral therapy (ART) in recent years, however, has

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offered hope of averting hundreds of thousands of deaths [2]. By April 2007, approximately 1.3 million people in the region were receiving treatment [3], and early pessimism concerning effective delivery of ART on a large scale using a simplified public health approach has largely proven unfounded. However, early mortality risk is much higher among patients treated in resource-limited settings compared with those treated in high-income countries [4,5].

Between 8 and 26% of patients receiving ART in sub-Saharan Africa die within the first year of treatment, with most deaths occurring in the first few months [4]. Baseline patient characteristics independently associated with early mortality include low CD4 cell count, advanced World Health Organization (WHO) clinical stage of disease, low body mass index, anaemia and male sex. In many programmes in Africa, median baseline CD4 cell counts are low (approximately 100–150 cells/μl) [4,6–11], and this has been highlighted as a key problem [4].

Although most existing studies from sub-Saharan Africa have examined short-term mortality outcomes and their association with baseline patient characteristics [4], some data suggest that risks of morbidity and mortality change according to the response to ART [11–13]. Data are now needed on longer term outcomes and, in particular, how mortality risk changes in association with CD4 cell count recovery. We hypothesized that even in those with rapid immune recovery, many patients with advanced immunodeficiency at baseline accrue considerable time with low CD4 cell counts, resulting in a high cumulative mortality risk. To examine these issues, we analysed prospectively collected data from patients with up to 5 years of follow-up in a large ART cohort in Cape Town, South Africa.

Methods

Antiretroviral treatment service

The ART service established in Gugulethu township in Cape Town in 2002 has previously been described in detail [6,12–14]. The district has a predominantly African population of over 300 000, the vast majority of whom live in conditions of low socioeconomic status. Ambulatory patients eligible for ART are referred to this service from a series of other primary care clinics in the township. During this study, the median time between enrolment of a patient in the service and initiation of ART was approximately 1 month. This permitted thorough preparation of patients for ART and screening and management of opportunistic infections and other comorbidities. The vast majority of patients with tuberculosis (TB) in this service have received at least 1 month of TB treatment prior to ART [15].

National ART guidelines were based on the WHO 2002 recommendations [16], providing treatment for those with a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count of less than 200 cells/μl. First-line ART comprised stavudine, lamivudine and a nonnucleoside reverse transcriptase inhibitor (predominantly efavirenz) and was supplied free of charge to patients. Treatment compliance was high, and the virological failure rate is approximately only 2% of patients per year [17]. All patients routinely received prophylaxis with trimethoprim-sulphamethoxazole both before and during ART.

Data collection

Blood CD4 cell count and plasma viral load measurements were done routinely at baseline and 4 monthly during ART within accredited laboratories. Detailed structured clinical and laboratory records were maintained for every patient visit from the time of enrolment in the programme. Data were transferred on a weekly basis to an electronic database. Patients requiring in-patient care were referred to a nearby 200-bed secondary hospital. Information on in-patient care was gained from discharge letters, hospital and laboratory records and postmortem examinations. Additional deaths and losses to follow-up were ascertained by active community-based follow-up by peer counsellors as previously described [13]. Patients were classified as lost to follow-up if they did not attend the clinic for a consultation or to collect medication for a period of 12 weeks. Collection of data on this study population for research purposes was approved by the Research Ethics Committee of the University of Cape Town; all patients enrolled gave written informed consent.

Data analysis

Data were analysed using STATA (StataCorp LP, College Station, Texas, USA). Data from all patients who initiated ART within the programme between September 2002 and June 2007 were included. Person-time of observation was accrued from the date of enrolment in the programme until either occurrence of death, loss to follow-up, transfer to another ART programme or censoring of observation in September 2007.

Person-time on ART was subdivided into 4-month intervals, each of which was defined by the CD4 cell count measurement at the start of the interval. In the event of missing CD4 cell data from the start of the interval (<5% of all intervals), we used the mean of the CD4 cell values immediately before and after the interval. Intervals were categorized into CD4 cell count strata (CD4cell-strata): 0–49, 50–99, 100–199, 200–299, 300–399, 400–499 and at least 500 cells/μl. Total person-time accrued within each of these CD4 cell-strata during follow-up was calculated.

Overall mortality rates and rates within CD4 cell-strata were calculated. Kaplan–Meier (product-limit) analyses were used to estimate cumulative mortality risk both in the overall cohort and stratified by baseline CD4 cell

count. We estimated the association between baseline risk factors, updated CD4 cell counts and viral load and the incidence of mortality using univariate and multivariate-mixed effect Poisson regression models.

The relationship between CD4 cell counts and duration of ART was illustrated using lowest smooth curves obtained from a series of locally weighted regressions with bandwidth of 0.8. Poisson base confidence intervals (CI) were calculated for per person-year incidence rates and binomial exact CIs for cumulative mortality rates. In other analyses, Wilcoxon rank-sum tests were used to compare medians, and all statistical tests are two-sided at alpha value of 0.05.

Results

Cohort characteristics and follow-up

During the study period, 2878 patients were enrolled in the programme. At the time data were censored, 2423 (84%) patients had started ART, and their baseline characteristics showed that most had advanced immunodeficiency (Table 1). Of 455 patients who did not receive ART, 298 (10%) had been deferred from the programme (due to ineligibility for ART, treatment refusal or loss to follow-up), 52 (2%) were currently preparing to start treatment and 105 (4%) had died before starting ART.

Individuals were followed up for up to 5 years, and a total of 3155 person-years of follow-up accrued during ART. With cohort expansion over time, the numbers of patients alive and receiving treatment after 1, 2, 3 and 4 years of treatment were 1426, 681, 262 and 104, respectively. Of those who started ART, 1856 (77%) were still alive and receiving treatment at data censorship, 232 (10%) had been lost to follow-up, 143 (6%) had been transferred out to another ART service and 192 (8%) had died. Those who died had low CD4 cell counts; at baseline, a median CD4 cell count of 78 cells/μl [interquartile range (IQR) 25–171; range 1–966)] at the time of death (Table 1). The proportions of patients with viral load suppression of less than 400 copies/ml at 12, 24, 36 and 48 months of ART were 90.5, 90.3, 93.3 and 90.5%, respectively. One-third of those who died had an undetectable viral load just prior to death (Table 1).

Mortality rates and cumulative mortality estimates

The mortality rate during the 1-month period prior to starting ART was very high [26.6 deaths/100 person-years; 95% CI 21.8–32.3]. The rate during months 0–4 of ART was also high (16.3 deaths/100 person-years; 95% CI 13.3–19.7) but decreased steeply thereafter, reaching a rate of 4.4 (2.8–6.4) deaths/100 person-years during 8–12 months of treatment. Mortality rates in the second, third and fourth years of ART were 2.6 (1.6–3.9), 0.7 (0.2–1.6) and 0.4 (0.1–1.6) deaths/100 person-years, respectively.

Cumulative mortality estimates over 48 months of ART were derived using Kaplan–Meier analyses (Fig. 1a). The mortality estimate at 12 months (8.4%) was approximately two-thirds of the overall estimate at 48 months (13.2%). Cumulative mortality at 48 months in patients with

Table 1. Characteristics of the patients starting antiretroviral treatment and of the patients who subsequently died during treatment.

Patient characteristics	Patients starting ART ($n=2423$)	Survivors ($n=2231$)	Deaths ($n=192$)
Median (IQR) age	33 (28–39)	32 (28–38)	36 (30–43)
Female patients	1624 (67)	1512 (68)	112 (58)
Baseline CD4 cells (cells/μl)			
Median (IQR)	101 (48–157)	104 (52–159)	57 (16–111)
0–49	618 (21)	531 (24)	87 (45)
50–99	574 (20)	531 (24)	43 (22)
100–149	549 (19)	516 (23)	33 (17)
150–200	423 (17)	406 (18)	17 (9)
≥200	259 (11)	247 (11)	12 (6)
Baseline log viral load			
≥5.0 log copies/ml	1052 (43)	946 (42)	106 (55)
WHO stage			
1 and 2	575 (24)	560 (25)	15 (8)
3	1395 (53)	1300 (58)	95 (50)
4	552 (23)	470 (21)	82 (43)
Updated CD4 cell count prior to death (cells/μl)			
Median (IQR)	–	–	78 (25–171)
0–99	–	–	107 (56)
100–199	–	–	45 (23)
≥200	–	–	40 (21)
Updated viral load prior to death			
≥400 copies/ml	–	–	128 (67)
Months ART at time of death			
Median (IQR)	–	–	7.8 (3.3–22.8)

Values show numbers (%) unless otherwise stated. ART, antiretroviral therapy; IQR, interquartile range.

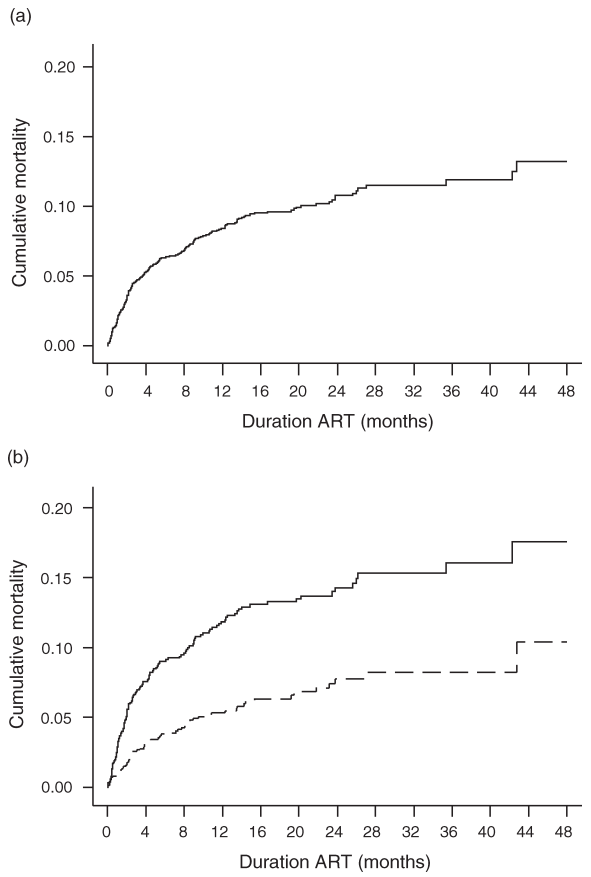

Fig. 1. Kaplan–Meier plots showing cumulative mortality risk over 48 months. (a) Cumulative mortality estimates for the whole cohort at 12 and 48 months of ART were 8.4 and 13.2%, respectively. (b) Cumulative mortality estimates at 12 and 48 months from enrolment were 11.6 and 16.7%, respectively, in those with baseline CD4 cell counts of less than 100 cells/μl (———) compared with 5.2 and 9.5% in those with baseline counts more than 100 cells/μl (- - - -).

baseline CD4 cell counts less than 100 cells/μl was approximately double that of patients with counts more than 100 cells/μl ($P < 0.001$) (Fig. 1b).

To permit comparison with data from other cohorts, mortality estimates after 48 months of ART were also determined for subgroups of patients. For those with WHO stages 1–3 and baseline CD4 cell counts of more than 100 cells/μl or less than 100 cells/μl, estimates were 6.3 and 12.6%, respectively. For those with WHO stage 4 disease and baseline CD4 cell counts of more than 100 cells/μl or less than 100 cells/μl, estimates were 20.1 and 24.8%, respectively.

Mortality rates and CD4 cell response to antiretroviral therapy

We next examined how mortality rates changed in relation to the CD4 cell response to ART. We categorized person-time of observation according to the CD4 cell count at the start of each 4-month follow-up interval. We

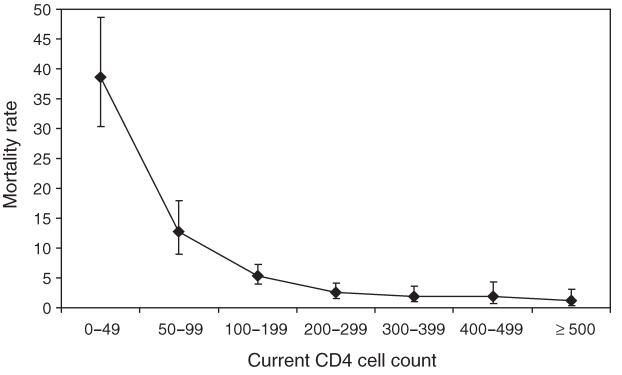

Fig. 2. Graph showing the mortality rates (95% confidence interval, deaths/100 person-years) according to updated CD4 cell counts during antiretroviral therapy. CD4 cell counts (cells/μl) were measured at baseline and 4 monthly during ART. Follow-up time intervals were defined by the CD4 cell count at the start of each interval and categorized into one of six different CD4 cell count strata. Cumulative person-time accruing within these strata was determined and used to derive the CD4 cell-stratum-specific mortality rates. Unadjusted mortality rates during person-time accrued within the 0–49, 50–99, 100–199, 200–299, 300–399, 400–499 and at least 500 cells/μl CD4 cell-strata were 38.6 (30.3–48.6), 12.8 (8.9–17.9), 5.4 (3.9–7.2), 2.7 (1.6–4.1), 2.0 (1.0–3.6), 2.0 (0.7–4.3) and 1.2 (0.3–3.2) deaths/100 person-years, respectively.

then calculated cumulative person-time accrued within a series of CD4 cell-strata and used these as denominators to calculate mortality rates stratified by updated CD4 cell counts. The relationship between updated CD4 cell counts and mortality rates was extremely strong (Fig. 2).

Risk factors for mortality during antiretroviral therapy

We examined crude and adjusted associations between mortality and patient baseline characteristics and updated viral load and CD4 cell counts. In crude analyses, older age, male sex, WHO stage 4 disease, updated CD4 cell counts and detectable updated viral load measurements were all significantly associated with mortality risk (Table 2). With the exception of male sex, each of these variables remained significantly associated in the multivariate model. However, updated CD4 cell counts were the variable most strongly associated with death. Those CD4 cell-strata lying below a threshold of 200 cells/μl were each associated with a more than two-fold greater adjusted mortality risk compared with the more than 500 cells/μl CD4 cell-stratum. Above a threshold of 200 cells/μl, small cumulative reductions in mortality rates occurred as CD4 cell counts increased further, but these differences did not reach statistical significance.

In another model, we excluded the first 4 months of observation during ART, so that baseline CD4 cell counts could be included as a separate variable in addition to updated CD4 cell counts. In this model, updated but not

Table 2. Risk factors for mortality among patients ($n = 2423$) receiving antiretroviral therapy.

	Crude association			Multivariate model		
Patient characteristics	IRR	95% CI	*P*-value	IRR	95% CI	*P*-value
Age	1.03	1.02–1.05	<0.001	1.04	1.02–1.05	<0.001
Male sex	1.72	1.29–2.29	<0.001	1.10	0.82–1.49	0.524
WHO stage						
1–3	1			1		
4	2.31	1.73–3.08	<0.001	2.28	1.70–3.07	<0.001
Cohort enrolment year						
1	1			1		
2	0.59	0.25–1.39	0.227	0.58	0.25–1.36	0.211
3	0.86	0.41–1.81	0.689	0.82	0.39–1.75	0.615
4	1.38	0.69–2.76	0.360	1.06	0.52–2.16	0.862
5	2.26	1.12–4.55	0.022	1.27	0.62–2.61	0.513
Updated CD4 cell count (cells/μl)						
>500	1			1		
400–499	1.59	0.45–5.63	0.460	1.41	0.40–5.01	0.597
300–399	1.60	0.50–5.11	0.425	1.45	0.45–4.64	0.533
200–299	2.16	0.74–6.34	0.158	1.66	0.56–4.91	0.358
100–199	4.37	1.57–12.17	0.005	2.59	0.90–7.42	0.076
50–99	10.44	3.71–29.43	<0.001	4.93	1.66–14.70	0.004
0–49	31.46	11.50–86.08	<0.001	11.63	3.95–34.29	<0.001
Updated viral load (copies/ml)						
<400	1					
>400	5.76	4.24–7.81	<0.001	2.06	1.37–3.09	<0.001

Age included as a continuous variable. CI, confidence interval; IRR, incidence rate ratio.

baseline CD4 cell counts were significantly associated with mortality risk. Together, these models indicate that the absolute CD4 cell count at any given time point is a key determinant of mortality risk.

CD4 cell count recovery during antiretroviral therapy

We next examined how the overall distribution of CD4 cell counts in the cohort changed over time. Figure 3 shows changes in the proportions of patients with CD4 cell counts lying below a series of thresholds during ART. The proportion of patients with a CD4 cell count less than 100 cells/μl decreased rapidly from 41% at baseline, reaching a plateau of approximately 2% after 12–18 months of ART. The proportion of patients with a CD4 cell count of less than 200 cells/μl also decreased from 89% at baseline and reached a plateau of approximately 10% of patients after 24–36 months of ART. Conversely, the proportion of patients with CD4 cell counts of more than 500 cells/μl steadily increased from 0% at baseline to approximately 50% after 48 months (Fig. 3). The slope of the 500 cells/μl contour indicates substantial immune recovery was ongoing after 4 years of treatment.

Cumulative person-time within low CD4 cell-strata and mortality risk

Having established the relationship between mortality risk and updated CD4 cell counts and having characterized CD4 cell recovery over time, we reasoned that mortality rates in the overall cohort would be strongly related to the cumulative person-time accrued within low CD4 cell-strata. For each sequential year of ART, we calculated the proportions of total person-time that were accrued within different CD4 cell-strata. During years 1, 2, 3 and 4 of ART, the proportions of person-time accrued at CD4 cell counts less than 100 cells/μl were

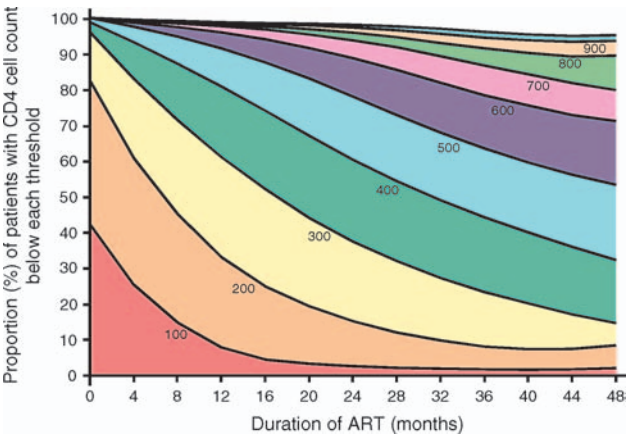

Fig. 3. Graph showing smoothed changes in distribution of CD4 cell counts in the cohort over time during 48 months of antiretroviral therapy. The proportions (percentage) of patients with CD4 cell counts lying below thresholds of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 cells/μl are shown. The proportions of patients with CD4 cell counts less than 100 and 200 cells/μl decreased steeply over the 1st and 2nd years of ART, respectively. The proportion of patients with CD4 cell counts more than 500 cells/μl steadily increased throughout follow-up, indicating ongoing immune recovery. Median CD4 cell counts at 0, 12, 24, 36 and 48 months of ART were 101, 261, 355, 417 and 483 cells/μl, respectively.

26, 4, 1 and 1%, respectively, and the proportions of person-time accrued at CD4 cell counts less than 200 cells/μl were 63, 25, 12 and 8%, respectively. These observations provide insight into the very high mortality rates in the first year of ART, which rapidly decreased to long-term rates that were much lower.

Baseline CD4 cell counts were strongly predictive of the cumulative person-time accrued within the lowest CD4 cell-strata. Those with the lowest baseline counts (<100 cells/μl) accrued 28 and 53% of person-time with CD4 cell counts of less than 100 and 200 cells/μl, respectively, compared with 1 and 28% of person-time for those with higher baseline counts (>100 cells/μl). Thus, patients with lower baseline CD4 cell counts had a much higher cumulative mortality risk (Fig. 1b).

Discussion

In this analysis, we examined the relationship between mortality risk and CD4 cell counts measured at baseline and 4 monthly during ART (updated CD4 cell counts) in a large cohort. Mortality risk decreased markedly in association with ART-induced CD4 cell recovery, but patients remained at substantially greater mortality risk during person-time accrued at CD4 cell counts below a threshold of 200 cells/μl. Compared with patients with the highest updated CD4 cell counts, patients with updated CD4 cell counts of less than 200 cells/μl had a more than two-fold greater adjusted mortality risk and those with updated counts of less than 50 cells/μl had a more than 10-fold greater adjusted mortality risk. Cumulative person-time accrued within CD4 cell-strata lying below 200 cells/μl explains both the high mortality observed during the first year of ART and the high long-term cumulative mortality risk among those with the lowest baseline CD4 cell counts. Early HIV diagnosis and timely initiation of ART before CD4 cell counts fall 200 cells/μl are required.

Despite virological suppression rates of more than 90% and excellent CD4 cell count recovery in this cohort (Fig. 3), cumulative mortality estimates after 48 months of ART were far greater than those observed in ART cohorts in high-income countries [18,19]. Similarly aged patients in the Dutch AIDS Therapy Evaluation Project (ATHENA) cohort, for example, who have sexually acquired HIV and baseline CD4 cell counts of less than 100 cells/μl have 5-year cumulative mortality estimates of 8% (AIDS patients) or 4% (non-AIDS patients) [18]. These compare to 4-year estimates of 24.8 and 12.6%, respectively, among similarly stratified patients in our cohort. Corresponding estimates from the ATHENA cohort for patients with baseline CD4 cell counts of 100–200 cells/μl were 4% (AIDS patients) and 2% (non-AIDS patients) compared with estimates of 20.1 and 6.3% in our cohort. These findings indicate that long-term cumulative mortality estimates are over three-fold higher in our cohort.

Our data extend the findings of the Antiretroviral Treatment in Lower Income Countries (ART-LINC) collaboration, which reported that after adjustment for baseline patient characteristics, mortality risk in the first year of ART was higher among patients treated in resource-limited settings compared with those treated in high-income settings despite similar immunological and virological responses to treatment [5,20]. Reasons underlying this have yet to be defined but may include differences in the spectrum of opportunistic pathogens and access to healthcare. The most common causes of early deaths in our cohort are TB, acute sepsis, cryptococcal meningitis, wasting syndrome and Kaposi's sarcoma [6,15,21]. Immune reconstitution disease has been speculated to contribute to early mortality in this setting, and our experience is that cryptococcal disease rather than TB is more important in this regard [6,22].

Existing studies from both high-income and resource-limited settings have largely focused on the relationship between mortality risk and baseline patient characteristics [4,6–11,13,18,19]. We extended these findings, showing that mortality risk changed markedly in relationship with CD4 cell count recovery during ART (Fig. 2) and that the absolute CD4 cell count at any given time point was the key determinant of mortality risk. However, above a threshold of 200 cells/μl, further increments in CD4 cell counts were associated with only minor additional reductions in mortality rates that did not reach statistical significance in this analysis.

Immune recovery in this cohort compares very favourably with that observed in ART cohorts in high-income countries [23]. The proportion of patients with CD4 cell counts less than 200 cells/μl decreased steeply to reach a plateau of approximately 10% after 24–36 months of ART (Fig. 3). In the long term, the minority of patients with updated counts less than 200 cells/μl contributed disproportionately to overall mortality rates, and this proportion may be an important factor underlying differences in mortality rates between cohorts. Such patients may have immunological nonresponse (approximately 10% of this cohort at 48 weeks [24]) or primary or secondary virological failure. A detectable viral load during follow-up was also an independent risk factor for death, highlighting the importance of maintaining virological suppression.

The slope of the 500 cells/μl CD4 cell count contour in Fig. 3 indicates that substantial immune recovery was ongoing after 48 months of ART. However, as mortality risk varied relatively little above a CD4 cell count threshold of 200 cells/μl, the steadily increasing proportion of patients with counts more than 500 cells/μl is unlikely to impact overall mortality rates substantially.

This may be more important, however, with regard to reducing risk of morbidity due, for example, to TB, which persists at high rates during ART in this setting [25].

Cumulative mortality risk was strongly related to the proportions of person-time accrued within different CD4 cell-strata. During the first year of ART, the proportions of person-time accrued within CD4 cell-strata lying below 200 cells/μl were large, and the mortality that accrued within this period was correspondingly high. Beyond 1 year of treatment, these proportions rapidly decreased, accounting for much lower mortality rates thereafter.

In multivariate analyses, mortality risk at any given time point was associated with updated CD4 cell count rather than the baseline value. Lower baseline counts were, however, predictive of greater proportions of person-time accrued within low CD4 cell-strata with high mortality risk. Thus, cumulative mortality estimates at 48 months were much higher among patients with baseline CD4 cell counts less than 100 cells/μl compared with those with higher baseline counts (Fig. 1b). Median baseline CD4 cell counts are low in most ART programmes in sub-Saharan Africa, and so many patients remain at high mortality risk for considerable periods.

These data indicate that patients should start ART before their CD4 cell counts fall below 200 cells/μl, though this only occurs in a small minority of patients in Africa at present. Earlier initiation of ART will require early HIV diagnosis and strengthening of longitudinal HIV care, with serial assessments of CD4 cell counts and clinical status to trigger initiation of ART at an appropriate time point. Eligibility criteria for ART in national programmes in the region need to be reconsidered. The policy in South Africa, for example, restricts ART to patients with WHO stage 4 disease (AIDS) or a CD4 cell count less than 200 cells/μl [26], which is not in keeping with current WHO guidelines [27]. Thus, current South African policy restricts the potential survival benefits that could be derived from ART.

Strengths of this study include the completeness of data, with less than 5% of CD4 cell count data-points missing, good ascertainment of outcomes and a low loss to follow-up rate. Previous data indicate that inadvertent misclassification of deaths as losses to follow-up in this cohort is infrequent [13]. This analysis is novel with inclusion of updated CD4 cell counts and viral load measurements as time-dependent covariates. A much larger cohort would be needed to detect any significant differences in mortality risk in higher CD4 cell-strata, however. Baseline characteristics of patients were typical of those accessing ART in other public sector programmes across sub-Saharan Africa. However, mortality and loss to follow-up rates were lower than those observed in many other programmes, and absolute CD4 cell-stratified mortality rates may not be representative of all programmes [4,28].

In summary, long-term cumulative mortality estimates in this South African cohort were approximately three-fold higher than those in cohorts in high-income countries despite excellent immunological and virological responses to ART. Mortality risk during ART was strongly associated with updated CD4 cell counts, and overall mortality was related to the proportions of person-time accrued within low CD4 cell-strata. Person-time accrued at CD4 cell counts less than 200 cells/μl must be minimized before and during ART. National HIV programmes should take measures to promote early diagnosis of HIV and initiation of ART before CD4 cell counts fall below 200 cells/μl.

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Usefulness of total lymphocyte count in monitoring highly active antiretroviral therapy in resource-limited settings

Motasim Badri and Robin Wood

Objective: To assess the usefulness of total lymphocyte count (TLC) for monitoring HIV-infected patients receiving highly active antiretroviral therapy.

Design: Observational cohort study.

Methods: Correlation between difference (Δ) from baseline at week 4, 8, 12 and 48 in TLC, CD4 cell count and viral load was determined in patients initiating HAART in phase III clinical trials between 1995 and 2001 at the HIV Clinical Research Unit, Somerset Hospital, Cape Town.

Results: The study included 266 patients. At weeks 4, 8, 12 and 48, median increase in TLC was 30, 52, 139 and 219 cells $\times 10^6$/l, median increase in CD4 cell count was 8, 48, 88, and 145 cells $\times 10^6$/l, and median decrease in viral load was -1.6, -2.2, -2.5 and -2.7 $\log_{10}$ copies/ml, respectively. The correlation between all pairs of ΔTLC and ΔCD4 cell counts was significant (r, 0.61; $P < 0.0001$), but between ΔTLC and Δ viral load it was not (r, -0.014; $P = 0.73$). However, the correlation between median viral load reduction and median increase in both ΔCD4 cell count (r, -0.96; $P < 0.0001$) and ΔTLC (r, -0.89; $P < 0.0001$) was significant. The slope of ΔCD4 cell count was [52.493 + 0.14(ΔTLC)]. Sensitivity and specificity of an increase or decrease from baseline in TLC for similar trend in CD4 cell count during follow-up were 83.4% and 87.3% respectively.

Conclusion: TLC correlated well with changes in CD4 cell count and at a group level with viral load changes. TLC may have a role in inexpensive monitoring of the immunological response to highly active antiretroviral therapy in a resource-constrained setting.

AIDS 2003, **17**:541–545

Keywords: HIV, total lymphocyte count, CD4 T lymphocyte count, viral load, HAART

Introduction

The survival benefits of highly active antiretroviral therapy (HAART) are well documented. However, owing to high cost, few in the developing countries currently have access to antiretroviral therapy (ART). Recent initiatives of the World Health Organization (WHO) for scaling up ART in resource-limited settings [1] will result in an increasing number of HIV-infected patients accessing ART. In well-resourced settings, commencement of ART is based predominantly on the presence of HIV-related symptoms and CD4 cell count. Laboratory capacity to measure CD4 cell count is limited in many areas with high HIV seroprevalence. The WHO has proposed a TLC of $< 1200 \times 10^6$/l as a substitute indication for initiating ART in resource-limited settings [1]. TLC is an inexpensive and useful marker for disease staging and predicting progression to AIDS or death in HIV-infected patients [2]. A high correlation between TLC

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and CD4 cell count both in asymptomatic and symptomatic HIV-infected patients has been observed [3].

Restoration and/or preservation of immunological function together with suppression of plasma viraemia are important goals of ART. Capacity to monitor plasma viral load is very limited in resource-constrained settings. Use of TLC as a surrogate for other expensive markers, such as the CD4 cell count or viral load, would result in substantial reduction in cost associated with managing HIV-infected patients.

We conducted an observational study to determine whether changes over time in CD4 cell count and plasma HIV RNA could be monitored by changes in TLC in a cohort of indigent patients accessing HAART through participation in phase III randomized clinical trials in a public healthcare facility in Cape Town, South Africa between 1995 and 2001.

Methods

This prospective observational study was conducted in the New Somerset Hospital HIV clinic, University of Cape Town; a major public health care facility dedicated for HIV-infected patients in Cape Town. This clinic provides care for patients referred from a wide range of primary healthcare facilities in Cape Town.

Subjects were recruited into the study from the ongoing clinical trials in the hospital, which have been described in detail elsewhere [4]. In brief, patients >16 years were recruited into the 12 trials conducted in the hospital between 1995 and 2001. Entry viral load and CD4 cell count requirements varied between studies. Patients were excluded if they presented with acute opportunistic infection, significant laboratory abnormalities, and active substance abuse or if they were treated with immune-modulating or systemic chemotherapeutic agents. Pregnant or lactating females were also excluded. All patients received at least three antiretroviral drugs: a non-nucleoside reverse transcriptase inhibitor with two nucleoside analogues; or three nucleoside analogues; or a protease inhibitor with two nucleoside analogues. Follow-up was repeated at 2–3-month intervals, or more frequently if clinically indicated. Total lymphocyte count (TLC), CD4 cell count and plasma HIV RNA were measured prospectively approximately every month. Plasma HIV RNA was determined by reverse transcriptase–polymerase chain reaction (Roche Amplicor HIV-1 RNA PCR assay version 1.5, Roche, Branchberg, New Jersey, USA), CD4 cell count by flow cytometry (Coulter, Hialeah, Florida, USA) and TLC by automated blood counter (CPICS, Hialeah, Florida, USA) on the same day the blood sample was obtained.

As all three parameters were non-normally distributed, when tested for normality using the Shapiro–Wilks' W test, association between all pairs of TLC, CD4 cell count and plasma HIV RNA was determined using Spearman's partial rank order correlation. Median change difference (Δ) in baseline TLC, CD4 cell count and viral load at weeks 4, 8, 12 and 48 were assessed using the Friedman ANOVA non-parametric test. Changes in CD4 cell count and plasma HIV RNA relative to TLC was determined using the slope of the correlation's scatter graph. Sensitivity and specificity of the absolute change (i.e., the absolute increase/decrease) in CD4 cell count and TLC from baseline at the different time points during follow-up (i.e., every 4 weeks) as well as for TLC $< 1250 \times 10^6$/l and CD4 cell count $< 200 \times 10^6$/l were calculated. The choice of a TLC $< 1250 \times 10^6$/l as surrogate for a CD4 cell count $< 200 \times 10^6$/l was based on the significant correlation observed previously in patients presenting to our clinic [2]. All analyses were carried out using STATISTICA software (release 6.6,Tulsa, Oklahoma, USA).

Results

The study population consisted of 266 patients receiving HAART. At screening, 155 failed to meet entry requirements. Mean age of patients was 34.5 years (SD, 9 years). 115 (44%) patients were female and 122 (46%) presented with WHO clinical stage 3 or 4 at their initial clinic visit. At inclusion, median CD4 cell count was 254×10^6/l [inter-quartile range (IQR), $140–364 \times 10^6$/l], median TLC was 1480×10^6/l (IQR, $1100–1830 \times 10^6$/l) and mean ($\log_{10}$) plasma HIV RNA was 5.4 copies/ml.

At weeks 4, 8, 12 and 48, median increase in TLC ($\times 10^6$/l) was 30, 52, 139 and 219, respectively. Median increase in CD4 cell count ($\times 10^6$/l) was 8, 48, 88, and 145, respectively and median decrease in plasma HIV RNA ($\log_{10}$ copies/ml) was -1.6, -2.2, -2.5 and -2.7, respectively. Median increase from baseline in TLC ($P < 0.0001$) and CD4 cell count ($P < 0.0001$), as well as median decrease in plasma HIV RNA ($P < 0.0001$) was significant (Fig. 1).

A significant correlation was observed between all pairs of ΔTLC and ΔCD4 cell count (r, 0.61; $P < 0.01$; Fig. 2a). The correlation between all pairs of Δ viral load (VL) and ΔCD4 cell count was significant (r, -0.26; $P < 0.0001$; Fig. 2c), but between all the pairs of ΔVL and ΔTLC it was not (r, -0.01; $P = 0.73$; Fig. 2b). However when the ΔTLC was log-transformed, the correlation reached significance (r, -0.011; $P = 0.03$; data not shown). The slope of ΔVL and ΔCD4 cell count relative to ΔTLC was [$-2.02 -$

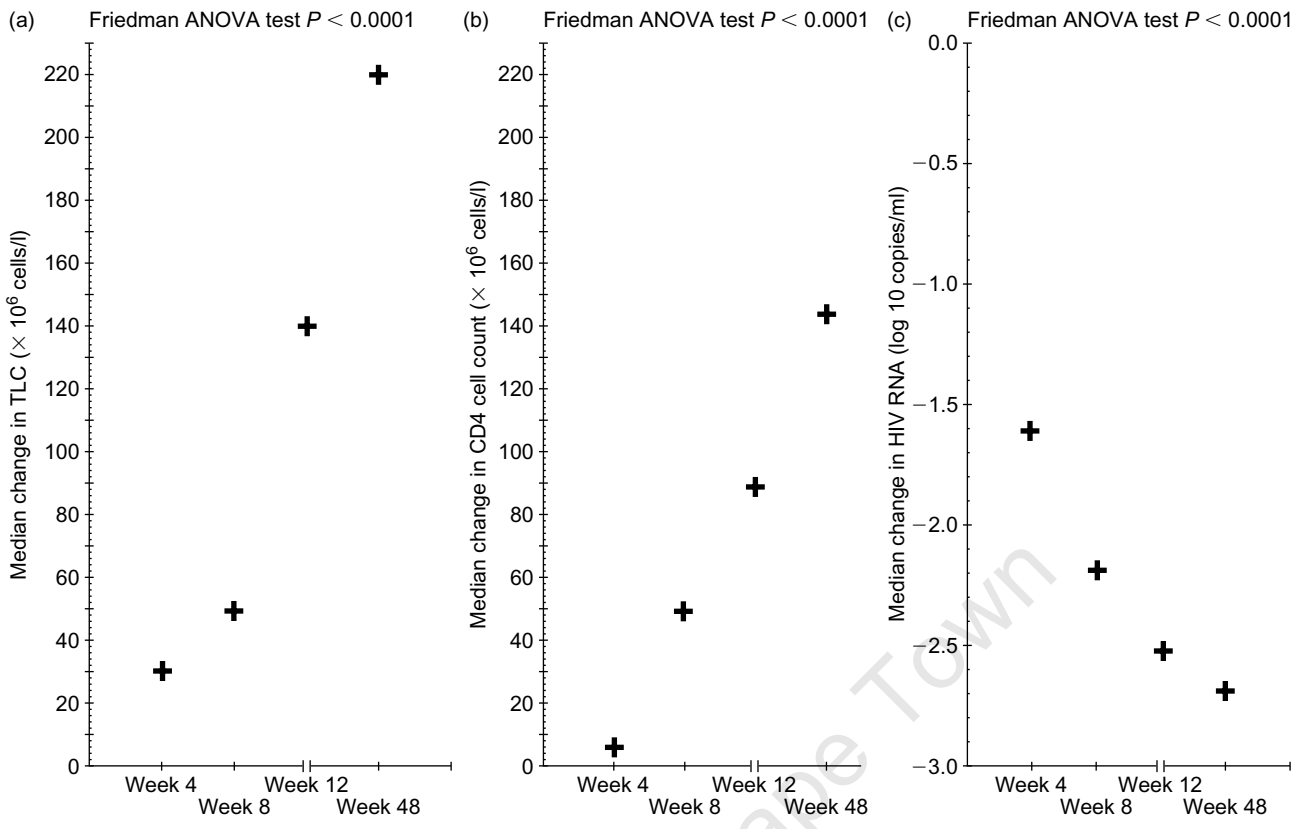

Fig. 1. Median change in TLC (a), CD4 cell count (b) and HIV RNA (c) at weeks 4, 8, 12, and 48.

0.00003(ΔTLC)] and [52.5 + 0.14(ΔTLC)] respectively, and the slope of ΔVL relative to ΔCD4 cell count was [−1.84 − 0.004(ΔCD4 cell count)].

Reduction in the median plasma HIV RNA at weeks 4, 8, 12 and 48 correlated well with median increase in both ΔCD4 cell count (r, −0.96; $P < 0.0001$; Fig. 2f) and ΔTLC (r, −0.89; $P < 0.0001$; Fig. 2e). The correlation between median increase in ΔCD4 cell count and ΔTLC was also significant (r, 0.98; $P < 0.0001$; Fig. 2d). The correlation between all the pairs ($P = 0.004$) and median increase ($P < 0.0001$) in ΔVL and ΔCD4 cell count were significantly greater than that between ΔVL and ΔTLC. The slope of median ΔVL and ΔCD4 cell count relative to ΔTLC was [−1.071 − 0.005(ΔTLC)] and [−0.07 + 0.67(ΔTLC)] respectively, and the slope of median ΔVL relative to CD4 cell count was [−1.69 − 0.008(ΔCD4 cell count)] (Fig. 2).

Sensitivity and specificity of an increase or decrease in TLC for a similar trend in CD4 cell count were 83.4% [95% confidence interval (CI), 81.5–85.1] and 87.3% (95% CI, 83.6–90.4), respectively. To validate our results, we calculated the sensitivity and specificity for the categorical values of the change from baseline in TLC of $< 1250 \times 10^6$/l and CD4 cell count of $< 200 \times 10^6$/l which were 99.4% (95% CI, 98.8–99.7) and 92.8% (95% CI, 89.6–95.1), respectively.

Discussion

This study evaluated the usefulness of TLC in monitoring patients initiating HAART by quantifying the association between improvement in TLC, CD4 cell count and plasma HIV RNA following use of HAART. Our findings indicate that TLC can be used as a reasonable surrogate for CD4 cell count. Although the association between change in TLC and plasma HIV RNA is weak at the individual level, TLC can be used to monitor plasma HIV RNA at the group level. In addition to the proposed use of TLC for initiating ART in resource-limited settings in the recent WHO guidelines [1], TLC may have a role in inexpensive monitoring of the immunologic response to HAART. Given the significant correlation and the relatively high sensitivity and specificity rates, in clinical practice TLC can aid health care providers in monitoring the immunologic response to HAART using the slope of the correlation ΔCD4 cell count [52.493 + 0.14(Δ TLC)]. For example, an increase from baseline of 1×10^6/l in TLC is equivalent to an increase of 192.5×10^6/l CD4 cell count.

Recent ART strategies are based on the objective of reducing plasma HIV RNA to below the detection limits of assays in order to stop or reverse the pathogenic process. Although usefulness of TLC as a

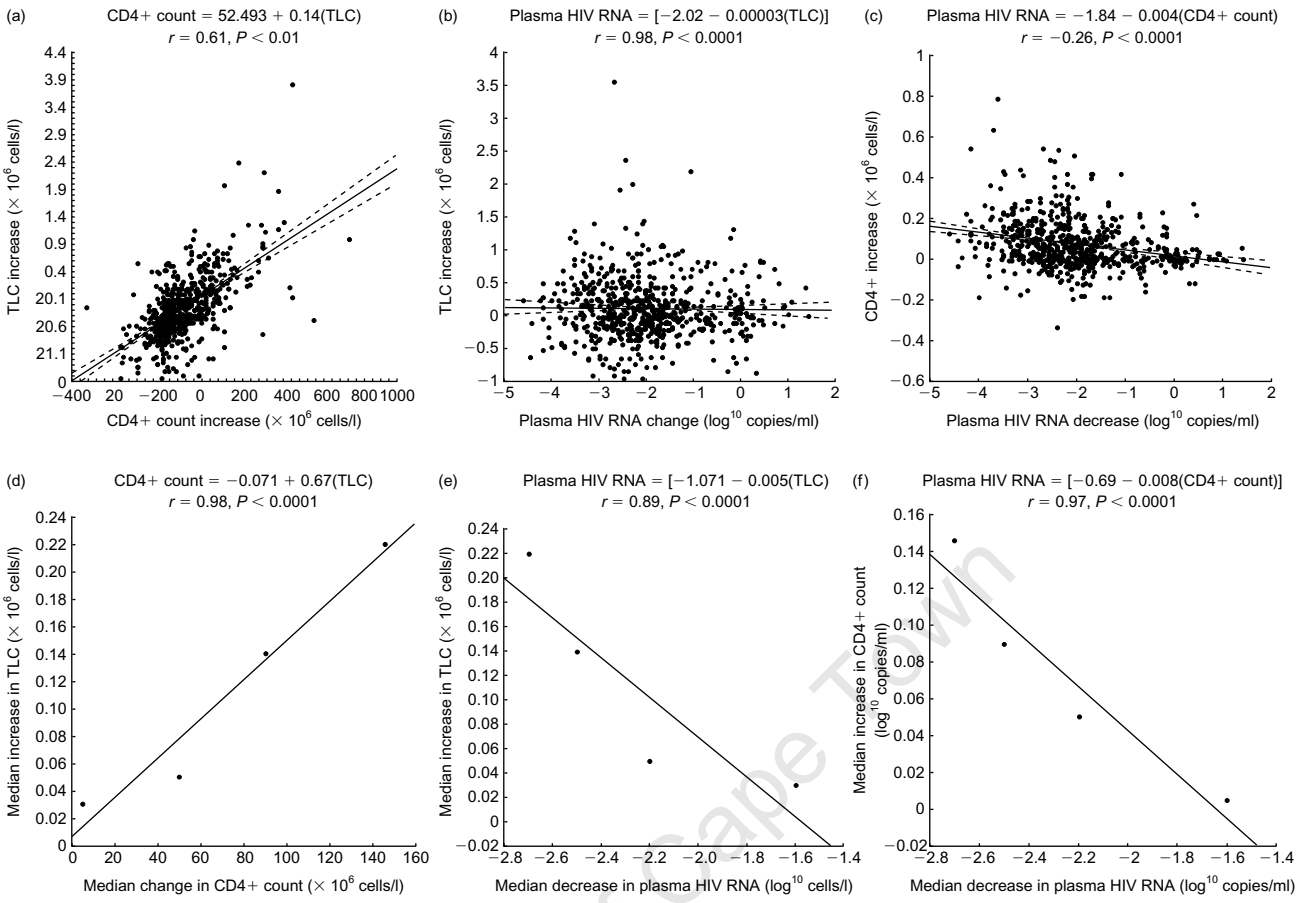

Fig. 2. Correlation between (a) change in TLC and CD4 cell count, (b) change in TLC and HIV RNA, (c) change in CD4 cell count and HIV RNA, (d) median change in TLC and CD4 cell count, (e) median change in TLC and HIV RNA, and (f) median change in CD4 cell count and HIV RNA.

surrogate for CD4 cell count have been studied previously, our study is the first to assess the association between changes over time in these two parameters and the plasma HIV RNA in patients receiving HAART. In a study from the UK, Beck *et al.* observed a high correlation (*r*, 0.76) between TLC and CD4 cell count [3]. The correlation in their study was consistently significant in asymptomatic patients (*r*, 0.64), symptomatic non-AIDS HIV-infected patients (*r*, 0.72) and AIDS patients (*r*, 0.73). Studies by Lai [5], Fournier *et al.* [6] and Pulido *et al.* [7] have also demonstrated significant correlation between TLC and CD4 cell count, particularly in patients with CD4 cell count $< 200 \times 10^6$/l. In addition, in a cohort of non-HAART users in Cape Town, Post *et al.* have demonstrated that TLC $< 1250 \times 10^6$/l and CD4 count $< 200 \times 10^6$/l predict similar progression to AIDS and mortality [2].

For HAART interventions to be cost-effective, targeting and timing are of utmost importance. More recently, the WHO proposed the use of a TLC of 1200×10^6/l as a substitute indication for ART treatment in resource-limited setting for CD4 cell count $< 200 \times 10^6$/l [1]. Our sensitivity and specificity analyses suggest that a TLC of 1250×10^6/l is a reasonable surrogate for evaluating the outcome of HAART in patients failing to achieve immunologic reconstitution to a CD4 cell count of 200×10^6/l. Our sensitivity estimate concur with recent study by Flanigan *et al.* from India (80%) [8].

The exclusion criteria for patients entering HAART trials were protocol-determined but may have introduced bias. The major causes for exclusion were acute opportunistic infection and significant laboratory abnormalities as substance abuse among our patients is minimal. Initiation of HAART at the same time as treatment for acute opportunistic infections results in high pill-burdens and added potential for adverse drug interactions. In addition, acute opportunistic infection may cause transient lymphopaenia [9]. However, despite these exclusions, 46% of the patients included in this study presented with WHO stage 3 or 4, indicating that our cohort represents patients with wide spectrum of immune suppression.

In conclusion, our findings indicate that in addition to its

use for initiating HAART, as proposed by the recent WHO guidelines, TLC can also be used as an inexpensive surrogate for monitoring the immunolgical response to HAART in resource-limited settings. This data is of particular relevance to sub-Saharan Africa where the laboratory infrastructure to perform CD4 cell and viral load measurement is frequently not available and current international initiatives for facilitating access to ART is increasing the number of patients requiring monitoring.

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BMC Infectious Diseases

Research article

Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting

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Abstract

Background: Viral load monitoring is not available for the vast majority of patients receiving antiretroviral therapy in resource-limited settings. However, the practical utility of CD4 cell count measurements as an alternative monitoring strategy has not been rigorously assessed.

Methods: In this study, we used a novel modelling approach that accounted for all CD4 cell count and VL values measured during follow-up from the first date that VL suppression was achieved. We determined the associations between CD4 counts (absolute values and changes during ART), VL measurements and risk of virological failure (VL > 1,000 copies/ml) following initial VL suppression in 330 patients in South Africa. CD4 count changes were modelled both as the difference from baseline (ΔCD4 count) and the difference between consecutive values (CD4 count slope) using all 3-monthly CD4 count measurements during follow-up.

Results: During 7093.2 patient-months of observation 3756 paired CD4 count and VL measurements were made. In patients who developed virological failure (n = 179), VL correlated significantly with absolute CD4 counts (r = - 0.08, $P = 0.003$), ΔCD4 counts (r = - 0.11, $P < 0.01$), and most strongly with CD4 count slopes (r = - 0.30, $P < 0.001$). However, the distributions of the absolute CD4 counts, ΔCD4 counts and CD4 count slopes at the time of virological failure did not differ significantly from the corresponding distributions in those without virological failure ($P = 0.99$, $P = 0.92$ and $P = 0.75$, respectively). Moreover, in a receiver operating characteristic (ROC) curve, the association between a negative CD4 count slope and virological failure was poor (area under the curve = 0.59; sensitivity = 53.0%; specificity = 63.6%; positive predictive value = 10.9%).

Conclusion: CD4 count changes correlated significantly with VL at group level but had very limited utility in identifying virological failure in individual patients. CD4 count is an inadequate alternative to VL measurement for early detection of virological failure.

Background

Access to antiretroviral therapy (ART) is expanding in low- and middle-income countries with over 2 million people receiving treatment by December 2006, representing 28% of the 7.1 million estimated to be in need [1]. Recent studies from sub-Saharan Africa have shown that ART is a cost-

effective public health intervention [2-4]. Over 1.3 million people in the region were receiving ART by December 2006 and yet more than 3.5 million further individuals remained untreated [1]. To date, early pessimism that ART could not be effectively delivered on a large scale in the region using a simplified public health approach has proven largely unfounded. However, lack of laboratory monitoring to identify patients failing treatment and requiring a switch in treatment regimen remains a critical issue.

Plasma viral load (VL) monitoring, the gold standard used in high-income countries for diagnosing virological failure, is not available in many resource-limited settings. Currently a single World Health Organisation (WHO)-recommended second-line regimen is the only therapeutic option available for HIV-infected patients in sub-Saharan Africa who develop virological failure during their first-line regimen [5]. Although these regimens are offered free of charge in the national ART programme in some countries, no further treatment options are typically available in the public sector thereafter. Sensitive and specific means for timely identification of treatment failure are therefore greatly needed to maximize the benefits of these limited drug options.

Routine VL monitoring in resource-limited settings requires significant infrastructure and expertise and remains prohibitively expensive in most settings. Other low-cost means of detecting virological failure must therefore be considered. Colebunders and colleagues, for example, proposed an algorithm based on clinical and treatment history and inexpensive laboratory indices such as haemoglobin level and total lymphocyte count [6]. However, when evaluated in a South African cohort, the sensitivity and specificity of the algorithm were unacceptably low [7]. WHO has recommended use of CD4 cell count measurements and clinical outcomes for monitoring ART in the absence of VL [5]. However, the clinical and CD4 cell count changes that are able to predict virological failure have not been identified.

When considering the utility of CD4 cell counts as a surrogate for virological failure, the critical issue is whether the variability in CD4 cell count measurements adequately reflects the variability in viral load. A number of previous observations suggest that this may be limited. Firstly, in a study of untreated patients in the USA, higher VLs were associated with greater rates of CD4 cell decline at a group level, but had minimal value for predicting the rate of CD4 cell decline in individual patients; only 4%–6% of the variability in CD4 cell losses could be explained by plasma VL [8]. Secondly, it is well recognised that a significant proportion of patients receiving ART have discrepant virological and immunological responses. Blood CD4 cell counts fail to increase in 5%–50% of patients receiving ART despite prolonged undetectable plasma VL. Conversely, marked increases in CD4 cell counts are observed in some patients despite incomplete virological suppression [9-14]. Thirdly, in a study from Botswana, initial blood CD4 cell count increases only had moderate discriminative ability for identifying those patients who successfully achieved VL suppression after starting ART [15]. Collectively these existing data suggest that CD4 cell counts have limited capacity to explain the variability of VL measurements at an individual level both in treated and untreated patients.

A number of studies have previously examined factors associated with virological treatment failure in high-income settings [16-23]. However, the practical utility of CD4 cell count measurements as a substitute for viral load monitoring has not been specifically assessed using rigorous analyses. Data relevant to ART programmes in resource-limited settings are especially needed. We therefore conducted an analysis of longitudinal data from the Cape Town AIDS Cohort (CTAC) in South Africa in which CD4 cell counts and VL measurements are routinely measured every three months. Using all data points measured during follow-up, we determined the association between VL measurements, risk of virological failure and CD4 cell counts analysed as either absolute values, changes from baseline (ΔCD4 count) or the difference between consecutive values (CD4 cell count slope). We were thereby able to assess the utility of CD4 cell counts to predict virological failure in a resource-limited setting.

Methods

Setting and study population

The Cape Town AIDS Cohort (CTAC) has been described in detail previously [24]. In brief, ART-naïve patients were referred to the cohort from a wide range of primary health care facilities in Cape Town to the adult HIV clinics affiliated with the University of Cape Town (UCT). Patients accessed ART through participation in multicentre phase III clinical trials at the New Somerset Hospital and the Desmond Tutu HIV Research Centre at UCT between 1996 and 2006. Participants gave informed consent and clinical trials protocols were approved by the UCT Clinical Research Ethics Committee. Enrolment criteria differed between the various trials but collectively encompassed patients with a wide spectrum of baseline blood CD4 cell counts, viral load and clinical stages. All patients received a minimum of three antiretroviral drugs: a non-nucleoside reverse transcriptase inhibitor and two nucleoside analogues; three nucleoside analogues; or a protease inhibitor with two nucleoside analogues.

Viral load was determined by reverse transcriptase-polymerase chain reaction (Amplicor®, Roche Molecular

Systems, Branchburg, New Jersey, USA) and CD4 counts were measured by flow cytometry (Beckman Coulter®, Miami, Florida, USA). Blood CD4 cell counts and plasma VL were measured every 2–3 months when patients were routinely reviewed. Clinical stage of disease was assessed using WHO criteria. Demographic data were recorded and the socioeconomic status of each patient was defined using the Cape Metropolitan Council suburbs composite index, which has been described previously [24].

Statistical analyses

In all analyses conducted in this study, virological suppression was defined by a VL of < 400 HIV RNA copies/ml following initiation of ART. The baseline CD4 cell count was that measured at the time that virological suppression was first achieved. Virological failure was defined as the first episode of viral load ≥ 1,000 HIV RNA copies/ml following previous successful VL suppression, confirmed by a second consecutive measurement. To investigate sensitivity thresholds, we also explored in separate analyses VL thresholds of > 400 and of > 10,000 HIV RNA copies/ml. Changes in CD4 cell count were reported in two ways: ΔCD4 was defined as the change in CD4 cell count from the baseline value and the CD4 count slope was defined as the difference between consecutive CD4 cell count measurements as determined by subtraction of the former value from the latter value.

Determinants of virological failure

The Wilcoxon matched pairs test was used to compare continuous variables and the χ^2 test for comparison of categorical variables. The Kaplan-Meier method was used to estimate the virological failure-free proportion. Cox proportional hazard regression models were fitted to identify factors associated with the likelihood of virological failure, using the SAS phreg procedure (SAS software version 8.2, SAS, Cary, NC, USA). In this analysis virological failure-free survival was defined as the time from the date of first virological suppression to when viral load was confirmed to reach > 1,000 copies/ml, death or last known clinic visit. Risk factors considered in the analysis were prevalent AIDS (prior to, or at the date of a first viral load < 400 HIV RNA copies/ml) and incident AIDS (occurring subsequent to the date of a first viral load < 400 HIV RNA copies/ml), socio-demographic variables (including age, socioeconomic status and gender), baseline CD4 cell count and follow-up CD4 cell count (categorized a priori as a < 100 or ≥ 100 cells/μl increase at any time-point during follow-up). Follow-up CD4 cell count measurements were modelled as a time varying covariate. At each time-point in the modelling process, the CD4 cell count value considered was the value recorded at that specific time-point, if available. Otherwise, the most recent recorded value (within 2–3 months) was considered. Variables significantly associated with the likelihood of occurrence of virological failure in univariate models ($P < 0.05$) were considered for inclusion in a multivariate model.

Association between CD4 count and viral load failure

Different strategies were employed to comprehensively assess the strength of the association between treatment-induced changes in CD4 cell count and virological failure. Firstly, for patients who failed virologically, we fitted three separate scatter-plots of all VL measurements ($\log_{10}$ copies/ml) done during follow-up and either the concurrently measured absolute CD4 counts, ΔCD4 count values or CD4 count slopes at each time-point. In these analyses the strength of association was assessed by calculating Pearson correlation coefficients.

For patients who developed virological failure, we next compared the distributions of CD4 cell values, ΔCD4 counts and CD4 cell slopes measured at the time of failure with the distributions of all data points from patients who did not develop virological failure. All CD4 cell count and viral load values included in these analyses were concurrently measured during follow-up. Data were included from the date of first viral load suppression until the date of development of virological failure or the date of last CD4 count measurement for those who did not fail virologically.

We next determined the association between the CD4 cell count slope and virological failure using a receiver operating characteristic (ROC) curve. The area under the ROC curve was assessed with the use of the C statistic. Sensitivity, specificity, positive predictive value, negative predictive value estimates were calculated, with 95% confidence interval (CI), using Clopper-Pearson exact method or Fleiss approximation as appropriate.

Results

Virological failure during follow-up

Of 360 patients who started ART during the study period, 330 (91.7%) achieved initial viral load suppression during follow-up and were therefore included in the analyses of virological failure. All treatment regimens incorporated at least 3 drugs; the numbers of patients receiving regimens based on triple nucleosides, a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor were 51 (15%), 115 (35%) and 164 (50%), respectively. Patients were followed for a median of 24.7 patient-months (IQR, 4.7–51.6) of observation. During this time, 15 (4.5%) patients died.

Overall, a total of 3756 paired CD4 cell count and VL measurements were made during 7093.2 patient-months of observation. 179 (54.2%) patients developed virological failure with an incidence of 30.3 (95%CI 26.2–34.2) cases per 100 patient-years. Virological suppression was

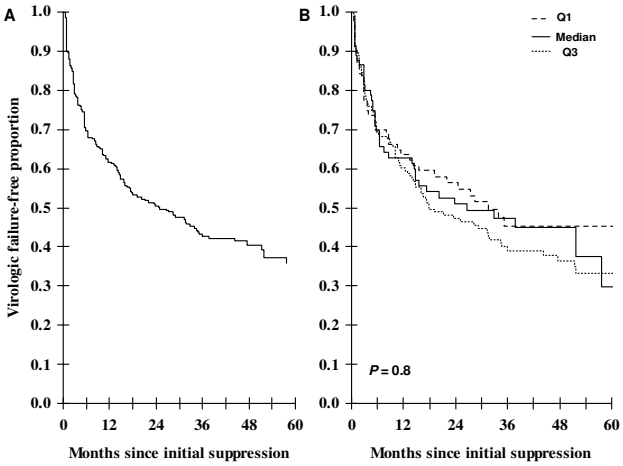


Figure 1
(A) Kaplan-Meier probabilities of virologic failure-free proportion. The numbers of patients followed up for 0, 12, 24, 36, 48 and 60 months were 330, 180, 123, 82, 39 and 26, respectively. (B) Kaplan-Meier probabilities of failure-free survival stratified by baseline CD4 cell count quartile range (median = 327; IQR = 205–435 cells/ul).

maintained in the remaining 151 (45.8%) patients. Kaplan-Meier analysis showed that risk of virological failure decreased with increasing duration of follow-up (Figure 1A). The median time to development of failure was 24.7 months.

Determinants of virological failure

The baseline clinical and socio-demographic characteristics are reported in Table 1. Groups of patients who did or did not develop virological failure were both composed of young adults with similar distributions of gender, socioeconomic status, and baseline CD4 cell count and clinical stage of disease. All had sexually acquired disease.

In univariate Cox proportional hazards regression models, none of the variables examined was significantly associated with the likelihood of virological failure (Table 1). These variables included follow-up CD4 cell count (Wald test P = 0.32), baseline CD4 cell count (Wald test P = 0.46), baseline WHO stage (Wald test P = 0.50), incident AIDS (Wald test P = 0.22), age (Wald test P = 0.09), gender (Wald test P = 0.63), and socio-economic status (Wald test P = 0.53). The lack of association with baseline CD4 cell count was further confirmed using a stratified Kaplan-Meier plot (Figure 1B).

In view of the lack of significant associations between patient characteristics and virological failure, multivariate analysis was not done. Collectively these data showed that development of virological failure was not associated with baseline patient characteristics, follow-up CD4 cell counts or the development of new AIDS-defining illnesses. In separate analyses, use of VL thresholds of > 400 and of > 10,000 HIV RNA copies/ml produced the same outcomes.

CD4 cell count changes and virological failure

We next examined in greater detail the associations between all viral load and CD4 cell count values measured concurrently during follow-up. Correlations between VL and absolute CD4 count, ΔCD4 cell counts and CD4 cell slopes were calculated for those patients who developed virological failure (Figure 2A–C). Significant correlations were observed between $\log_{10}$ VL and both absolute CD4 cell count values (r = - 0.08, P < 0.01), ΔCD4 cell count (r = - 0.11, P < 0.01), and most strongly with CD4 count slope (r = - 0.30, P < 0.001). This suggests that the

Table 1: Baseline demographic and clinical characteristics of the cohort studied (N = 330 patients).

Characteristic	Not failed (n = 151)	Failed (n = 179)	*P*-value
Gender			
Male	84(56)	93(52)	0.51
Female	67(44)	86(48)	
Age [median years(IQR)]	34(29–40)	32(27–38)	0.15
Socioeconomic status			
High status	68(45)	88(49)	0.45
Low status	83(55)	91(51)	
Baseline CD4 cell count (cells/μl)			
Median (IQR)	331(193–467)	323(208–431)	0.74
< 200	40(27)	38(21)	0.28
200–350	41(27)	62(35)	
> 350	70(46)	79(44)	
WHO stage			
Stage 1&2	74(49)	83(46)	0.82
Stage 3	59(39)	71(40)	
Stage 4	18(12)	25(14)	

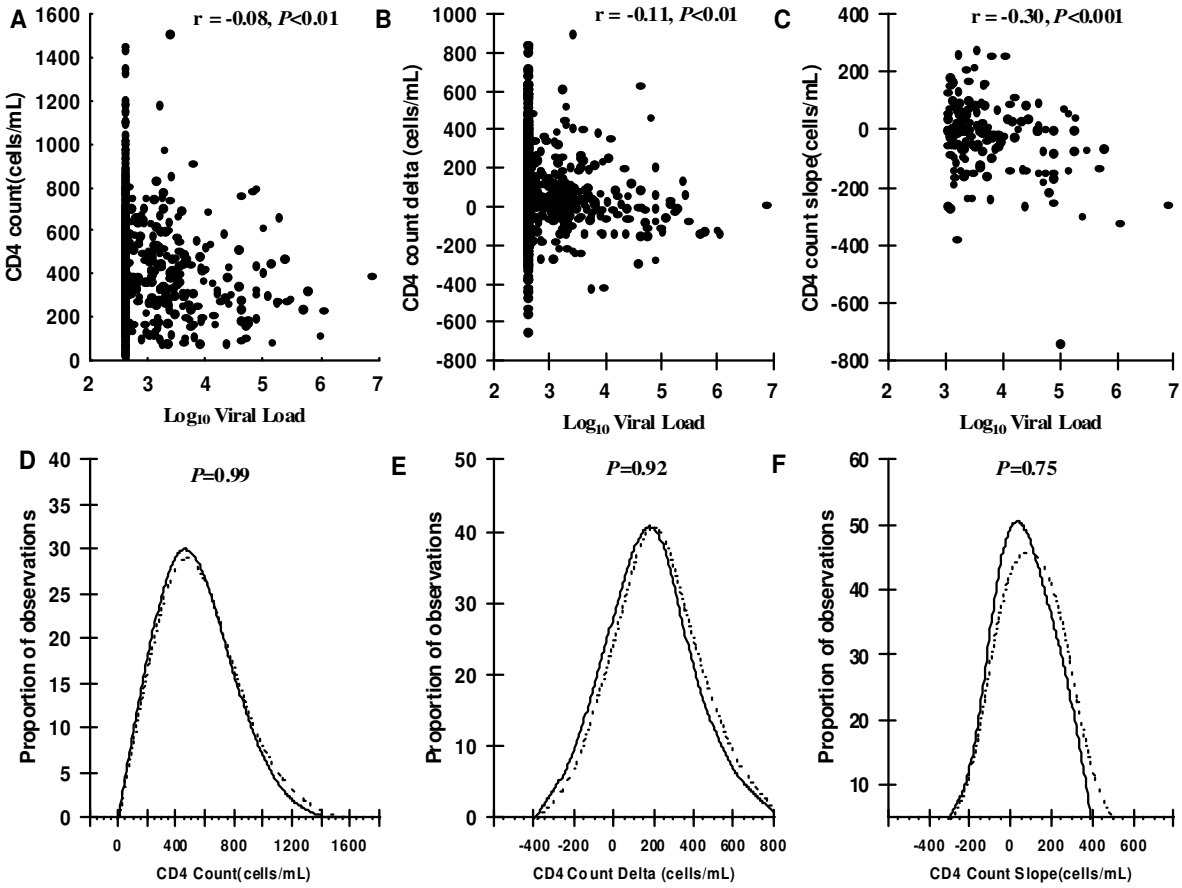


Figure 2

Scatter plots of (A) absolute CD4 cell count, (B) ΔCD4 cell count (change in CD4 count from baseline) and (C) CD4 cell count slope (difference between consecutive CD4 count measurements) and corresponding viral load values (log_{10} copies/ml) measured in patients who developed virological failure. Distributions of (D) absolute CD4 counts, (E) ΔCD4 counts and (F) CD4 count slopes of patients (n = 179) at the time of virological failure (dashed lines) compared to the distribution of measurements of all patients (n = 330) at all time-points when viral load remained suppressed (solid lines).

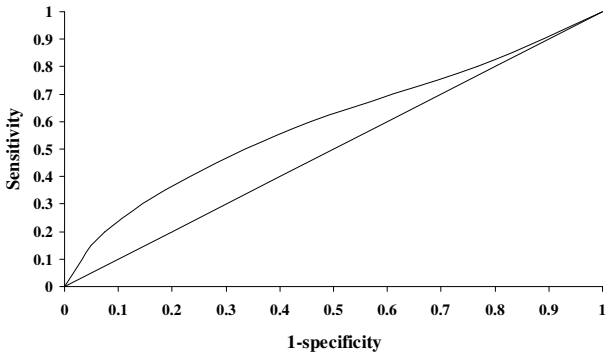
CD4 count slope at a given time-point would be the strongest indicator of the likelihood of virological failure.

CD4 cell counts measured at the time of virological failure were also compared with the distribution of all CD4 cell count measurements obtained from patients who did not develop failure (Figure 2D–F). These analyses showed that the distributions of absolute CD4 counts, ΔCD4 counts and CD4 count slopes did not significantly differ comparing values during virological failure to values during viral load suppression (P = 0.99, P = 0.92 and P = 0.75, respectively).

Since CD4 slopes were the parameter most strongly associated with log_{10} VL among those who developed virological failure, we fitted a receiver operating characteristic curve (ROC) using data from all the patients to examine this association further (Figure 3). This analysis showed that the predictive value of CD4 cell slope for virological failure was poor. The area under the ROC curve was 0.59 and the sensitivity, specificity, positive predictive and negative predictive values were all low (Figure 3).

CD4 cell counts among patients who did not achieve virological suppression

Virological suppression was not achieved by 30 (8.3%) of the total of 360 patients treated in this cohort during follow-up and were therefore not included in the above analyses. Separate analysis of data from these patients showed that a significant correlation was similarly observed



Negative CD4 cell count slope		Virological failure	
		Yes	No
Yes		95	775
No		84	1352
Sensitivity (95%CI)	Specificity (95%CI)	Positive Predictive Value (95%CI)	Negative Predictive Value (95%CI)
53(45.4-60.1)	63.6(61.5-65.6)	10.9(9.0-13.2)	94.2(92.8-95.3)

Figure 3
Receiver Operating Characteristic (ROC) curve assessing the association between a negative CD4 cell count slope (ie a falling CD4 count) and virological failure (area under the curve = 0.59).

between all measurements of absolute CD4 counts and the corresponding $\log_{10}$ VL measurements (r = - 0.25, $P < 0.0001$) (Fig 4A). However, the distributions of the ΔCD4 counts and CD4 count slopes in this group did not differ significantly from those observed among the 151 patients who achieved and maintained virological suppression during follow-up ($P = 0.87$ and $P = 0.25$ respectively) (Fig 4B–C). This showed that CD4 cell counts were also a poor correlate of viral load among patients who did not achieve viral load suppression.

Discussion

Early detection of virological failure is important for optimal management of HIV-infected patients receiving ART. Patients who continue to receive a failing regimen are at risk of immunological failure, morbidity and death. Moreover, accumulation of multiple antiretroviral drug resistance mutations may compromise the response to future drugs and fuel the spread of primary drug resistance within communities. Since VL monitoring is not available in most resource-limited settings, we investigated the utility of CD4 cell count measurements for predicting virological failure in a cohort of South African patients. Baseline absolute CD4 cell counts as well as clinical and socio-demographic characteristics were not predictive of virological failure. Analyses of longitudinal data from those who developed virological failure revealed that absolute CD4 cell counts and CD4 cell count changes (ΔCD4 cell counts and CD4 cell count slopes) were significantly correlated with viral load measurements at a group level. However, subsequent analysis showed that none of these methods of analysing CD4 cell counts could be used to identify individual patients at the time they developed

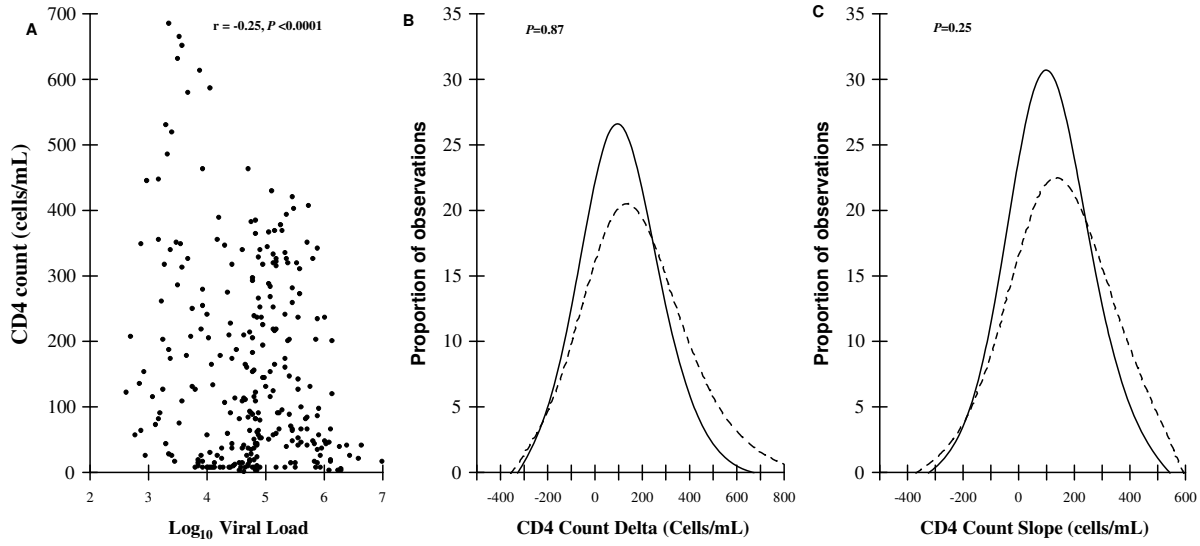


Figure 4
Scatter plot of the absolute CD4 count (cells/μl) and corresponding HIV RNA viral load ($\log_{10}$ copies/ml) for the subset of patients (n = 30) who did not ever achieve virological suppression during ART (A). The distributions of ΔCD4 counts (B) and CD4 cell count slopes (C) of this group are compared with that of patients who achieved and maintained virological suppression throughout the study period (n = 151).

virological failure. Since the distributions of CD4 cell counts and CD4 cell count changes among those with virological failure did not differ significantly from those of patients who maintained virological suppression, these could not be used to provide a clinically useful means for individual patient assessment for virological failure.

A unique feature of our study is that we used a novel modelling approach that accounted for all CD4 cell count and VL values measured during follow-up from the first date that VL suppression was achieved. Some previous studies have modelled the difference between the CD4 cell counts measured at initiation of treatment and at a single arbitrary point during ART defined *a priori*. Other studies assessing factors associated with virological failure did not account for all CD4 cell count measurements performed during follow-up [15-23]. Neither of these approaches fully evaluates CD4 cell count dynamics during ART, increasing the potential for misclassification bias. Our analytic approach also differs in that we modelled virological failure as the end-point of interest rather than virological treatment success as reported elsewhere. We used a VL > 1,000 copies/ml to define treatment failure consistent with local protocols. However, the same outcomes were obtained using thresholds of > 400 and > 10,000 copies/ml, which is consistent with previous data from this setting [25].

Baseline CD4 cell count was not predictive of virological failure in this ART-naïve population. However, in patients who developed virological failure, absolute CD4 cell count measurements, ΔCD4 cell counts and CD4 cell count slopes during ART each correlated significantly with VL measurements taken at the same time-points. Of these three parameters, the CD4 cell count slope was the most strongly correlated. This indicates that the rate of increase or decrease of CD4 cell count at a given time-point was the parameter that was most strongly associated with current VL. However, the distributions of ΔCD4 cell count and CD4 cell count slope values were very broad even among patients who maintained virological suppression. This suggests that considerable fluctuations in CD4 cell counts occur among patients despite sustained virological control. When these distributions were compared with the distributions of data from patients who had current virological failure, they almost completely overlapped. This demonstrated that absolute CD4 cell counts and CD4 cell count changes could not be used to identify patients who have developed virological failure. These findings were further corroborated by the observation that the distributions of CD4 cell count changes in the 30 patients who never achieved virological suppression were also broadly overlapping with the distributions of data from those who maintained virological suppression.

To investigate these associations further, we focussed on the use of CD4 cell count slopes since this was the parameter most strongly associated with VL at a group level. However, ROC curve analysis confirmed that use of CD4 slopes provided very poor test characteristics for predicting virological failure. The specificity and sensitivity of a negative CD4 cell count slope was low, showing that this parameter was not of practical utility in this clinical setting. Furthermore, the data show that a negative CD4 cell count slope could not even be used as a screen to identify those at high risk of virological failure as a means of rationing scarce viral load monitoring resources.

A strength of this study is that patients were closely followed in a multicentre clinical trials unit with strict protocols for regular clinical and laboratory monitoring every 2–3 months, leading to reliable identification of virological failure. As soon as a VL > 1,000 copies/ml was first detected, confirmatory viral load testing was done. The cohort characteristics were diverse and so the data are not only relevant to those with advanced immunodeficiency. Despite differing cohort characteristics, follow-up procedures and analytic approaches, our data are consistent with and extend previous studies that have found a poor association between CD4 cell counts and the development of virological failure [8,15].

We acknowledge the limitations of this study. An important potential limitation is that all patients studied were ART-naïve. Therefore these findings may not be generalisable to treatment-experienced patients. Our patients participated in international multicentre clinical trials. Their experience may differ from that of patients accessing treatment in a community-based setting. We do not have good assessments of treatment compliance although the mechanism underlying virological failure is unlikely to affect the relationship between CD4 cell counts and viral load. Despite a limited cohort size, follow-up in this study was prolonged, a substantial proportion developed virological failure and the number of paired CD4 cell counts and VL measurements was large.

Conclusion

In conclusion, we have shown that although changes in CD4 cell count correlated significantly with VL at a group level, they had very poor predictive value when being used to assess individual patients. Thus, CD4 cell count measurements cannot be used as a substitute for virological failure monitoring. Rigorous cost benefit analyses are required to further evaluate use of VL monitoring in this setting. Furthermore, there is a great need for development of simplified techniques to measure VL and for exploration of alternative low-cost assays for monitoring [26].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All the authors participated in the design of the analyses and the writing and revising of the manuscript. MB did the analyses.

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Nevirapine to prevent HIV transmission via breastfeeding

We, the co-principal investigator from India on the study of 6-week extended-dose nevirapine (July 26, p 300)[1] and co-workers, are at variance with the way the paper has been presented and interpreted. The study team has concealed crucial parts of the findings under complex statistical analysis and disguising adjustments.

First, the simple conclusion is that nevirapine given to infants for 6 weeks did not significantly reduce HIV transmission, compared with single-dose nevirapine, at the primary endpoint of 6 months. This endpoint was determined before the study began and was based on the fact that nevirapine given for the baby's first 6 weeks (when HIV transmission via breastfeeding is very high) would last for another 10–14 days owing to its long half life, and that it takes between 10 days and 4 months for the baby to become PCR positive after entry of the virus.[2] Therefore the fact that PCR positivity was lower at 6 weeks of age in the extended-treatment group than in the single-dose group does not reflect the effect of 6 weeks of treatment at all.

Second, about 40% of infants in both groups had grade III or IV side-effects (rashes, neutropenia, or elevated aminotransferases). The study team has not shown the duration of neutropenia, which could have varied with single-dose and 6-week regimens. One can imagine the clinical consequences of prolonged neutropenia.

Third, the study team has analysed all "intention to treat cases (modified)". However, if one wants to study the efficacy of a drug, one should analyse the per-protocol population. The intention-to-treat population is more relevant for safety studies. Use of the per-protocol population for efficacy analysis has been questioned since some patients might discontinue a drug if they do not feel better and would thus get eliminated, skewing the results. But this subjective factor is not relevant to the present study, where the primary endpoint is objective—ie, becoming HIV positive. If both intention-to-treat and per-protocol populations had been studied together and both gave identical results, confidence in the trial would have increased.

Finally, the study team conclude that nevirapine ought to be given for longer than 6 weeks. This recommendation is inappropriate, since the risk of transmission via breastmilk is highest in the early weeks of life and falls as age advances.[3,4] It is wrong that a drug which has not shown significant benefit and which has serious toxic effects in 38·4% of babies should be tried for longer and at a time when the risk of transmission has decreased. No drugs might be required at all, or some other strategy such as weaning foods might help.

It might be more prudent to follow WHO/UNICEF guidelines for developed countries and to make formula feeding safe, sustainable, acceptable, and affordable for mothers in developing countries. The cost, training, and manpower involved in giving 6 weeks of nevirapine to such women could be better used to educate them and supply milk substitutes in a hygienic form, thus bringing them in line with the developed world.

MAP was the principal investigator in India. PMB is a laboratory director and had a vital role nearly throughout the trial. NAK was part of the study team and did pharmacokinetic studies on nevirapine. We declare that we have no conflict of interest.

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Monitoring of antiretroviral therapy in low-resource settings

Using a computer simulation model, Andrew Phillips and colleagues (April 26, p 1443)[1] assessed the cost-effectiveness of three strategies for monitoring patients on antiretroviral therapy (ART) in resource-limited settings: viral load, CD4 cell count, or clinical events. We agree that lack of access to laboratory monitoring should not hinder expansion of ART access. However, we are concerned that the effectiveness of viral load monitoring might have been underestimated and that national policy makers might use these data to dismiss the use of laboratory monitoring strategies altogether.

Poor treatment adherence is the most common reason for virological failure and, although potentially reversible, Phillips and colleagues make the assumption that all patients with a single viral load of greater than 400 copies per mL should switch to second-line ART. We have previously reported from a South African programme[2] that three-quarters of patients whose viral load increases to more than 400 copies per mL during ART subsequently achieve virological suppression after implementation of a targeted ART adherence intervention. As a result, only 2% of patients per year in this programme develop persisting virological failure requiring second-line ART.[2]

Thus, instead of triggering high rates of switching to second-line therapy, viral load monitoring can actually be

used to promote adherence, thereby retaining patients on first-line therapy and minimising drug costs. Additionally, viral load monitoring might indirectly enhance retention of patients by identifying those with poor adherence who are in turn at risk of loss to follow-up.[3]

Finally, viral load measurements are valuable for monitoring and evaluation, permitting strengthening of ART programmes. In conclusion, viral load monitoring has far greater use than as a simple trigger for second-line therapy.

We declare that we have no conflict of interest.

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Recent international debate has focused on the need for CD4 count monitoring, viral load testing, or both, for HIV treatment in resource limited-settings. Andrew Phillips and colleagues address this question using a computer simulation model,[1] and conclude that the "benefits of viral load or CD4 cell count monitoring over clinical monitoring alone are modest."

We have concerns with the main results in table 4 and the methods that underlie them. First, Phillips and colleagues do not eliminate "weakly dominated" strategies—ie, strategies that are more expensive and incrementally less cost-effective than more expensive strategies—which is a crucial step in cost-effectiveness analysis.[2,3] They calculate the incremental cost-effectiveness ratio of the viral load monitoring strategy by erroneously comparing it with a weakly dominated strategy—CD4 decline from peak. Had they eliminated the weakly dominated strategies, the final results would be as shown in our table.

	Life-years	Costs (US$)	Cost per life-year gained (US$)
Viral load >500 copies per mL	10·56	4055	3494
New WHO 3/4 event	10·22	2867	2662
Multiple WHO 3 events/new WHO stage 4 event	10·01	2308	927
New WHO stage 4 event	9·75	2067	..

Table: **Phillips and colleagues' table 4, with weakly dominated strategies eliminated**

Second, the incremental cost-effectiveness ratio for viral load monitoring, when calculated correctly (US$3494 per life-year gained), should be compared with an external standard (eg, 3× the per-capita gross domestic product[4]). Reporting it as "not the most cost-effective" does not allow a policy maker to compare its value with any "willingness to pay" threshold.

When calculated correctly, the results also suggest that all CD4 monitoring strategies are weakly dominated by clinical monitoring strategies. These results are at odds with our published work[5] which finds that CD4 monitoring is cost-effective in Côte d'Ivoire compared with clinical monitoring alone. This finding calls into question whether the value of CD4 monitoring has been underestimated and, therefore, whether the incremental value of viral load monitoring has been overestimated by comparison.

We declare that we have no conflict of interest.

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In their Article on the outcomes of monitoring HIV-infected individuals by use of clinical observation, CD4 counts, or viral load,[1] Andrew Phillips and colleagues claim that expanding access to treatment should proceed without attention to diagnostic monitoring, since outcomes are only trivially worse with observation alone. This is an interesting analysis, but it ignores the true benefit of CD4 monitoring: determining the timing of treatment initiation.

Outcomes for individuals who start treatment in Africa are substantially worse than in developed countries, especially in the first year after treatment initiation.[2] There are two main factors that drive this gap: higher incidence of opportunistic infections, especially tuberculosis, and a lower CD4 count at treatment initiation. Waiting until the CD4 drops to the levels Phillips and colleagues assumed is avoidable and changing. CD4 counts at treatment initiation have been rising

Original article

HIV type-1 clade C resistance genotypes in treatment-naive patients and after first virological failure in a large community antiretroviral therapy programme

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Background: This study aimed to evaluate HIV type-1 (HIV-1) drug resistance pretreatment and in those failing first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART) in South Africa.

Methods: This was an observational cohort. Genotypic resistance testing was performed on treatment-naive individuals and those failing first-line ART (confirmed HIV-1 RNA >1,000 copies/ml) from public sector clinics in Cape Town (2002–2007). Resistance profiles and mutations relative to timing of known virological failure were examined.

Results: In total, 230 patients (120 treatment-naive and 110 with virological failure) were included: 98% had clade C virus. Among treatment-naive patients, prevalence of primary resistance was 2.5% (95% confidence interval 0.0–5.3). Three patients had one significant reverse transcriptase mutation: K65R, Y181C and G190A. Among treatment-experienced patients, 95 (86%) individuals had therapy-limiting NNRTI mutations, including K103N (55%), V106M (31%) and Y181C (9%). The M184V mutation was the most common mutation, found in 86 (78%) patients. In total, 10 (9%) patients had the K65R mutation. More individuals tended to develop thymidine analogue mutations when sampling occurred after 6 months of detected therapy failure (10/31 [32%] individuals) compared with those who had genotyping before 6 months (15/79 [19%] patients; *P*=0.246).

Conclusions: Prevalence of primary resistance in a sample of ART-naive clade C HIV-1-infected individuals in South Africa was low during the study period. Patients failing first-line ART most often developed resistance to NNRTIs and nucleoside reverse transcriptase inhibitors, the two drug classes used in first-line therapy. Viral load monitoring in this setting is crucial and individual genotypes in those failing first-line therapy should be considered.

Introduction

More than 3 million individuals now have access to antiretroviral therapy (ART) in low- and middle-income countries (LMIC) [1]. Delivery of ART on this scale has required utilization of a public health approach in which standardized, rather than individualized, regimens are prescribed to very large numbers of HIV type-1 (HIV-1)-infected individuals [2]. At present, the majority of individuals in these countries are initiating first-line therapy with a non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTIs) [3]. In addition to those receiving ART for treatment, many women receive nevirapine (NVP) and/or zidovudine (AZT) for prevention of mother-to-child HIV-1 transmission (PMTCT) [4].

Second-line ART, based on a boosted protease inhibitor with two NRTIs, is several-fold more expensive than the first-line regimens [1]. Although the proportion of patients receiving second-line therapy is

presently estimated to be 4%, this is increasing by 3% per annum [5]. In LMIC the decision of when to change to a second-line regimen is frequently delayed, as it is often based on clinical or immunological criteria in the absence of viral load measurement [3,6]. Rational choice of the NRTI component of second-line therapy should be based on patterns of resistance developed during first-line therapy [7].

Much concern was expressed during the initial phase of expanded access to ART that 'antiretroviral anarchy and viral mayhem' might follow the widespread use of ART in LMIC [8]. However, despite the large scale of PMTCT and ART rollout, there has been little published data detailing resistance either prior to or within large scale ART programmes. The effect of the widespread use of single-dose NVP for PMTCT on primary resistance patterns of those entering ART programmes has not yet been widely characterized [9].

Furthermore, most data on viral mutations developing in patients on ART are from industrialized countries where HIV-1 subtype B is prevalent, whereas viral subtypes in LMIC are frequently non-B, and non-B subtypes might have different pathways to viral resistance [10–12]. Data on resistance patterns in both treatment-naive and treatment-exposed clade C subtype are limited [13–21]. Our objective was to describe the resistance genotype patterns in both ART-naive individuals and in those with first virological breakthrough while on first-line NNRTI therapy in the public sector ART programme in South Africa.

Methods

Study samples

Treatment-naive samples

Staff at the Desmond Tutu HIV Centre in Cape Town, South Africa, drew 30 samples per annum for genotyping from treatment-naive HIV-1-positive individuals between 2003 and 2006, resulting in 120 samples available for the current analysis. These individuals were from two periurban resource-poor communities in Cape Town. HIV-1-infected individuals attending HIV clinics at either of the two sites were asked to donate a sample on a first-come first-serve basis beginning in April each year until 30 samples had been collected. None of these individuals had been exposed to any ART, including PMTCT, at the time of sampling.

Treatment-experienced samples

All samples were from individuals failing first-line therapy in public sector ART clinics in the greater Cape Town area between 2002 and 2007. Eight clinics provided samples. HIV-1-positive individuals in these clinics could access ART if they had a $CD4^+$ T-cell count <200 cells/μl or with WHO stage 4 clinical disease. First-line ART consisted of stavudine (d4T) and lamivudine (3TC), with an NNRTI (efavirenz or NVP) [6]. Pregnant women who did not yet qualify for ART were commenced on AZT at 34 weeks and given a single dose of NVP in early labour [4]. For this study, virological breakthrough was defined as the first time a viral load was noted to be >1,000 copies/ml while on ART, and virological failure was defined as two consecutive viral loads >1,000 copies/ml.

Laboratory testing

Viral load and $CD4^+$ T-cell counts were monitored 4- to 6-monthly according to local protocol [4,6]. Viral load assays were done using the branch DNA hybridization technique (Bayer HIV-1 RNA 3.0 assay). Genotypic analyses of the reverse transcriptase and protease sequences of HIV-1 DNA were completed using either the Trugene® HIV-1 (Bayer Healthcare, Leverkusen, Germany) or GeneSeq™ (Monogram Biosciences, South San Francisco, CA, USA). The International AIDS Society (IAS)–USA list of mutations was used to determine which mutations might be related to drug resistance [22]. Mutations noted in the IAS–USA listing that were not noted in this data group were not listed in the results. The Stanford University HIV Resistance Database Genotypic Resistance Interpretation Algorithm was used to determine the possible drug resistance patterns per genotype [23].

The majority of samples were sourced from the Hannan Crusaid Treatment Centre (Cape Town, South Africa); this cohort has been described previously [13–15]. Excess plasma from the 4-monthly scheduled visits was frozen and stored. All individuals who reached a viral load of >1,000 copies/ml and who had an available stored plasma sample at the time of confirmation of virological failure were analysed for HIV-1 genotype. The dates of treatment initiation, first observed virological breakthrough (>1,000 copies/ml) and confirmation of virological breakthrough (the date of the sample used for genotype analysis) were recorded.

Other samples were sourced from other ART sites in the Western Cape. Clinicians were asked to refer patients with a previously noted viral load of >1,000 copies/ml on first-line therapy to the study site for one-off genotype sampling. The dates of treatment initiation, first virological breakthrough (>1,000 copies/ml) and confirmation of virological breakthrough were recorded.

Demographic data (age, gender and disease stage) were recorded for all individuals with genotype results, as was viral load and $CD4^+$ T-cell count at the time of genotypic sampling. Mutations considered related to the function of the HIV-1 reverse transcriptase and protease enzymes were recorded.

Statistical analyses

Demographic and baseline data were described using medians and proportions as appropriate. Baseline

characteristics described by interval data were compared using non-parametric statistics for data not normally distributed. The 95% confidence intervals (CIs) around resistance mutation prevalence were constructed using the normal approximation of the binomial distribution. Among patients failing ART, we examined the association between timing of genotyping and resistance profile.

Results

Treatment-naive patient samples

Samples from 120 ART-naive HIV-1-infected individuals were included in the current analysis (Table 1). The median age of the cohort was 31 years (interquartile range [IQR] 25–38) and 63% were women. The median CD4$^+$ T-cell count at the time of sampling was 262 cells/μl (IQR 149–405) and the median viral load was 4.88 $\log_{10}$ copies/ml (IQR 4.29– 5.23). In total, 117 (98%) samples were clade C; the other 3 samples were clade B and were known to be collected from men who have sex with men [24].

The individual genotype results for the treatment-naive sample group are shown in Table 2 (Figure S1 in the Additional file). There was very little variation in the reverse transcriptase gene. One individual (0.8%) had a K65R mutation, denoting probable reduced sensitivity to tenofovir and abacavir, and three (2.5%) had the V118I mutation. Despite this cohort having no prior NNRTI exposure, there were two (1.7%) individuals with single NNRTI mutations, one Y181C and one G190A. In contrast to the reverse transcriptase, in the protease inhibitor region there were a number of mutations that occurred frequently, although these were not expected to cause drug resistance. The most frequent protease mutations were L89I/M (89%), H69K (88%), L63P (52%) and M36I (87%). In addition, >10% had mutations at loci 20 (17%), 74 (10%) and 77 (18%).

Treatment-experienced patient samples

In total, 119 individuals attending the Hannan Crusaid Treatment Centre between September 2002 and December 2007, who were taking first-line therapy, experienced virological breakthrough. Six individuals who had failed a protease-inhibitor-based first-line regimen were excluded from the analysis. Stored samples were not available for 34 individuals and genotype results were obtained for the remaining 79 individuals. Samples from an additional 31 individuals with the same failure criteria were received from seven other public sector antiretroviral clinics bringing the total number of genotypes available from individuals failing first-line therapy to 110.

The demographics of the treatment-experienced group were similar to that of the treatment-naive cohort. Their median age was 32 years (IQR 28–35) and 70% were women. The median CD4$^+$ T-cell count was significantly lower than in the treatment-naive group, at 192 cells/μl (IQR 128–288; P=0.003) and the median log viral load at time of sampling was significantly lower at 4.02 $\log_{10}$ copies/ml (IQR 3.61–4.76; P<0.001). The median time from treatment start date to initial detected virological breakthrough (>1,000 copies/ml) was 271 days (IQR 177–525), and that from first detected virological breakthrough to the time of confirmation and sampling for genotype was 97 days (IQR 31–195). Overall, 79 (72%) people had their repeat sample within 180 days of their initial raised viral load and 39 (28%) people after 180 days.

There were many more reverse transcriptase mutations in the treatment-experienced samples than in the

Table 1. Demographic characteristics and laboratory results for treatment-naive and first virological failure groups in a sample from Cape Town, South Africa

Characteristic	Treatment-naive	Virological failure	*P*-value
Total samples, *n* (%)	120 (100)	110 (100)	–
Mean age, years (IQR)	31 (25–38)	32 (28–35)	0.535
Female gender, *n* (%)	75 (63)	77 (70)	0.588
Median CD4$^+$ T-cell count, cells/μl (IQR)	262 (149–405)	192 (122–283)	0.003
Median viral load, $\log_{10}$ copies/ml (IQR)	4.88 (4.29–5.43)	4.02 (3.61–4.76)	<0.001
HIV-1 clade C, *n* (%)	117 (98)	56 (97)[a]	0.966
HIV-1 clade B, *n* (%)	3 (2)	2 (3)[a]	1.00
Treatment regimen			
d4T+3TC+EFV, *n* (%)	–	78 (67.2)	–
d4T+3TC+NVP, *n* (%)	–	18 (15.5)	–
AZT+3TC+EFV, *n* (%)	–	7 (6.0)	–
AZT+3TC+NVP, *n* (%)	–	6 (5.2)	–

The viral load and CD4$^+$ T-cell count presented for the first-time virological failure group are those at the time of second consecutive viral load >1,000 copies/ml, a median of 97 days from initial viral load >1,000 copies/ml. [a]Of 58 available. AZT, zidovudine; EFV, efavirenz; HIV-1, HIV type-1; IQR, interquartile range; d4T, stavudine; NVP, nevirapine; 3TC, lamivudine.

Table 2. Genotype results in a sample of ART-naive patients in Cape Town, South Africa

NRTI mutations				NNRTI mutations				PI mutations			
Ref	Loci	AA	*n* (%)	Ref	Loci	AA	*n* (%)	Ref	Loci	AA	*n* (%)
K	65	R	1 (0.8)	Y	181	CY	1 (0.8)	L	10	IVF	6 (5.0)
V	118	I	3 (2.5)	G	190	ACE	1 (0.8)	G	16	E	2 (1.6)
–	–	–	–	–	–	–	–	K	20	RT	20 (16.6)
–	–	–	–	–	–	–	–	M	36	I	104 (86.7)
–	–	–	–	–	–	–	–	M	46	I	1 (0.8)
–	–	–	–	–	–	–	–	I	47	IV	1 (0.8)
–	–	–	–	–	–	–	–	I	50	V	1 (0.8)
–	–	–	–	–	–	–	–	I	54	V	1 (0.8)
–	–	–	–	–	–	–	–	L	63	HLPSTV	62 (51.7)
–	–	–	–	–	–	–	–	H	69	K	108 (90)
–	–	–	–	–	–	–	–	A	71	ATV	3 (2.5)
–	–	–	–	–	–	–	–	G	73	S	3 (2.5)
–	–	–	–	–	–	–	–	T	74	S	12 (10)
–	–	–	–	–	–	–	–	V	77	I	22 (18.3)
–	–	–	–	–	–	–	–	LV	82	AF	1 (0.8)
–	–	–	–	–	–	–	–	L	89	IM	107 (89.2)

Overall *n*=120. Prevalence of wild-type virus was 114 (95%). Mutations noted in the International AIDS Society–USA listing that were not noted in this group are not listed here. AA, amino acid; ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; Ref, reference.

Table 3. Genotype results in a sample of patients with virological failure to first-line ART in Cape Town, South Africa

NRTI mutations				NNRTI mutations				PI mutations			
Ref	Loci	AA	*n* (%)	Ref	Loci	AA	*n* (%)	Ref	Loci	AA	*n* (%)
M	**41**	**ML**	**1 (0.9)**	A	98	G	4 (3.6)	L	10	IVF	7 (6.4)
K	65	R	10 (9.0)	L	100	I	2 (1.8)	I	13	V	8 (7.3)
D	**67**	**N**	**14 (13)**	K	101	EP	18 (16)	G	16	E	9 (8.1)
T	69	DN	2 (1.8)	K	103	N	60 (55)	K	20	RT	30 (27)
K	**70**	**KR**	**4 (3.6)**	V	106	M	34 (31)	D	30	N	1 (0.9)
L	74	L/I/V	1 (0.9)	V	108	IV	13 (12)	L	33	FV	2 (1.8)
V	75	IM	3 (2.7)	E	138	A	2 (1.8)	M	36	I	95 (86)
V	118	I	2 (2.7)	V	179	DV	6 (5.5)	L	63	HLPSTV	66 (60)
M	184	V	86 (78)	Y	181	CY	11 (10)	H	69	K	103 (94)
T	**215**	**FSY**	**9 (8.1)**	Y	188	HL	9 (8.1)	G	73	S	1 (0.9)
K	**219**	**EQ**	**5 (4.5)**	G	190	ACE	22 (20)	T	74	S	14 (13)
K	238	T	2 (1.8)	P	225	H	15 (14)	V	77	I	17 (15)
–	–	–	–	F	227	L	7 (6.4)	LV	82	AF	1 (0.9)
		M	230	L	8 (7.3)	L	89	IM	90 (82)		

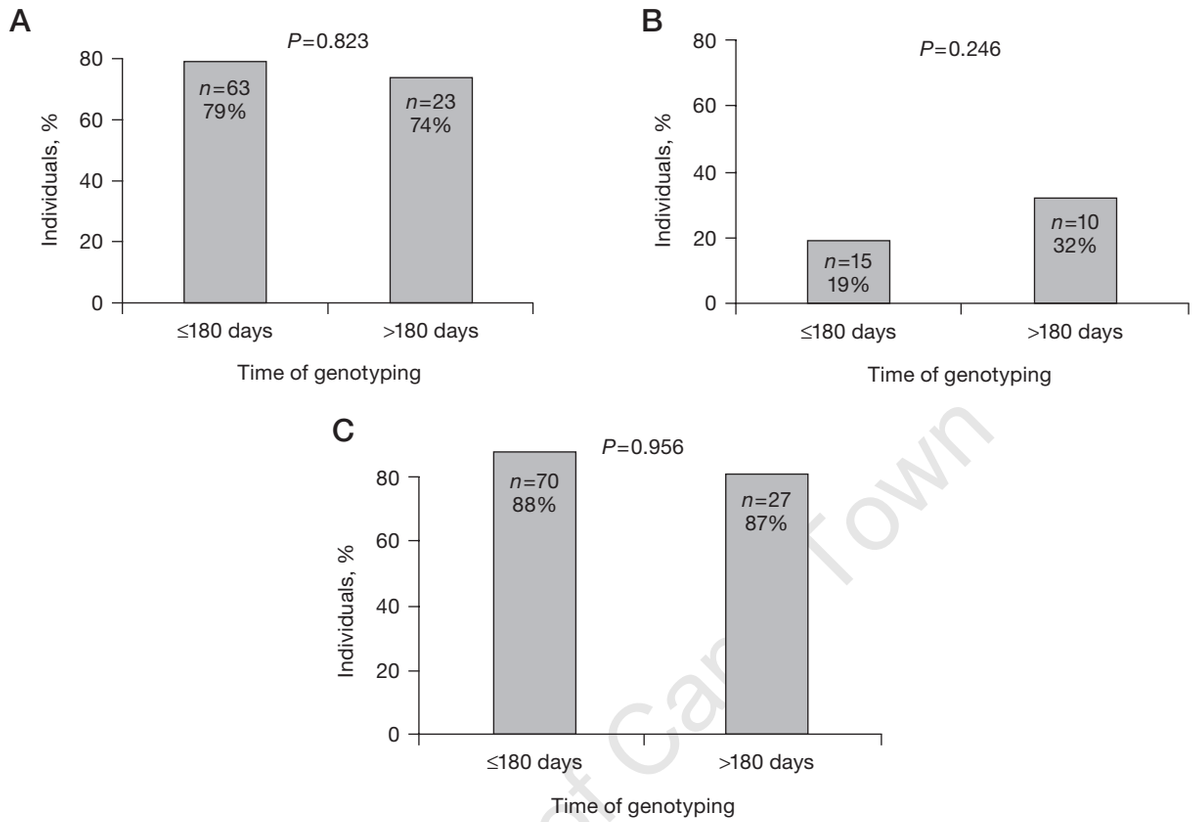
Overall *n*=110. Mutations noted in the International AIDS Society-USA listing that were not noted in this group are not listed here. Text in bold indicates thymidine analogue mutations (TAMs). Prevalence of wild-type virus was 7 (6.4%). AA, amino acid; ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; Ref, reference.

treatment-naive samples (Table 3 and Figure S2 in the Additional file): 91 (83%) individuals had ≥1 mutations limiting susceptibility of NRTIs and 97 (88%) individuals had ≥1 therapy-limiting NNRTI mutations. The M184V, conferring resistance to 3TC and emtricitabine (FTC), was the most common single mutation (*n*=86 [78%]) and emerged rapidly in failure (Figure 1A). According to the genotypic resistance interpretation algorithm four (4.0%) more people were likely to have intermediate resistance to 3TC and FTC because of the presence of K65R (Table 4) [23]. Seven (6.4%) of the samples had no reverse transcriptase mutations.

A total of 10 (9.0%) individuals, all taking d4T, had developed the K65R mutation, limiting future use of didanosine (ddI), tenofovir and abacavir, without having had exposure to any of these medications (Table 4). In this group, those with the K65R did not have a significantly higher mean viral load than those without [25]. Six (5.5%) individuals had both the K65R and the M184V mutations.

Figure 1.

[AU: Please provide title for Figure 1]



The proportion of individuals with either **(A)** the M184V mutation **(B)** thymidine analogue mutations (TAMs) or **(C)** non-nucleoside reverse transcriptase inhibitor mutations by time between initial virological breakthrough (first viral load >1,000 copies/ml) and failure (at the time of the second consecutive viral load >1,000 copies/ml). Individuals were divided as those whose genotype (taken at the time of virological failure) was completed on or before 180 days (*n*=79) from breakthrough or after 180 days (*n*=31). None of the differences were significant, although a trend toward more TAMs with more time on failing therapy was noted.

Overall, 25 (23%) individuals had a total of 33 thymidine analogue mutations (TAMs). Those with TAMs had a median viral load of 4.32 $\log_{10}$ copies/ml (IQR 3.47–4.71) compared with a median of 4.01 $\log_{10}$ copies/ml (IQR 3.47–4.71) in those without TAMS at the time of genotype sampling (*P*=0.896) [26]. Only five (4.5%) individuals had ≥1 (TAM). Figure 2 describes the proportion of those with non-TAM resistance that had also developed TAMs in the reverse transcriptase gene. There were relatively few individuals with resistance to either 3TC, that is, individuals presenting with the M184V mutation (*n*=6 [5.4%]), or NNRTIs (*n*=20 [18%]) alone. These individuals also had few TAMs, and no sample had ≥1 TAM. Over two-thirds (*n*=75 [68%]) of individuals had a combination of 3TC and NNRTI resistance. Of these, 21 (28%) had TAMs and it was only in these samples that 2 or 3 TAMs were noted.

More TAMs were noted in those individuals who had failure confirmed >180 days after initial virological breakthrough (Figure 1B). A total of 17 TAMs were noted in 15/79 (19%) individuals whose genotype was completed within 6 months, compared with 16 TAMs noted in 10/31 (32%) individuals whose genotype was completed after 6 months (*P*=0.246). Multivariate logistic regression modelling of factors associated with acquiring a TAM demonstrated that for every 20 unit increase in $CD4^+$ T-cell count at time of genotyping, the reduction in risk of developing TAMs was 9% (odds ratio 0.91, CI 0.83–0.99; *P*=0.035). Age, gender, time from failure to sample, viral load and NRTI used did not affect the acquisition of TAMs. There was no significant difference in the number of TAMs generated by the specific thymidine analogue taken, whether AZT (4/13 [31%] individuals) or d4T (28/97 [29%] individuals; *P*=0.917). Susceptibility to AZT and d4T remained high in this group (Table 4).

Development of NNRTI resistance occurred rapidly and these mutations were the most common noted in this group (Figure 1C). A total of 70/79 (88%) individuals whose genotype was completed within 6 months

Table 4. Expected resistance patterns according to the Stanford University HIV Resistance Database genotypic resistance interpretation algorithm in a sample of patients with virological failure to first-line ART in Cape Town, South Africa

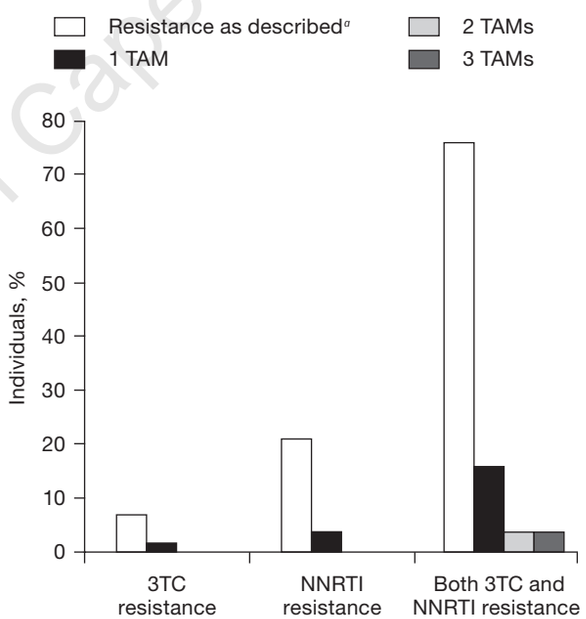
ART	Susceptible, *n* (%)	Possible low-level resistance, *n* (%)	Low-level resistance, *n* (%)	Intermediate resistance, *n* (%)	High resistance, *n* (%)
Lamivudine/emtricitabine	22 (20)	–	–	4 (4.0)	86 (78)
Abacavir	20 (18)	55 (50)	15 (14)	20 (18)	–
Zidovudine	98 (89)	1 (1.0)	6 (5.5)	3 (2.7)	2 (1.8)
Stavudine	87 (79)	6 (5.5)	12 (11)	5 (4.5)	–
Didanosine	76 (69)	9 (8.1)	9 (8.1)	14 (13)	2 (1.8)
Tenofovir	97 (88)	1 (1.0)	4 (3.6)	8 (7.3)	–
Efavirenz	10 (9.0)	2 (1.8)	–	13 (12)	85 (77)
Nevirapine	10 (9.0)	2 (1.8)	1 (1.0)	4 (4.0)	93 (85)
Etravirine	10 (9.0)	15 (14)	49 (45)	32 (29)	4 (4.0)
Protease inhibitors	93 (84)	16 (15)[a]	1 (1.0)[b]	–	–

Overall *n*=110. [a]Fourteen individuals with possible low-level resistance to nelfinavir alone; two with possible low-level resistance to fosamprenavir and tipranavir. [b]One individual with low-level resistance to atazanavir, fosamprenavir, lopinavir and saquinavir and intermediate resistance to indinavir and nelfinavir. ART, antiretroviral therapy.

had NNRTI mutations, compared with 27/31 (87%) individuals whose genotype was completed after 6 months (*P*=0.956). In total, 97 (88%) individuals had ≥1 therapy-limiting NNRTI mutations (Table 3), including K103N (55%), V106M (31%) and Y181C (10%), and probable drug susceptibility according to the genotypic interpretation algorithm was poor (Table 4) [23]. In total, 31 (28%) individuals had 1 NNRTI mutation, 46 (42%) had 2 NNRTI mutations, 16 (15%) had 3 NNRTI mutations and 3 (3%) samples had as many as 4 NNRTI mutations. One (0.9%) individual had 6 NNRTI mutations. The Y181C mutation emerged more frequently in those failing NVP (8/25 [32%]) than efavirenz (3/85 [3.5%]; *P*=0.0004). There was no significant difference in the emergence of K103N whether NVP (9/25 [36%]) or efavirenz (51/85 [60%]) was taken (*P*=0.229) [27]. There was no significant difference in the emergence of V106M by drug. This mutation was seen in 8/25 (32%) people on NVP and 26/85 (31%) people on efavirenz (*P*=0.923). The ratio of V106M/K103N in patients failing EFV therapy was 0.5.

The protease gene had similar mutations to those noted in the treatment-naive population. The median number of mutations was four (IQR 3–5). The most frequent protease mutations remained M36I (86%), L63P (60%), H69K (94%) and L89I/M (82%), similar to the consensus sequence noted for clade C (differing amino acids compared with clade B subtypes at positions M361, R41K, H69K and L89M) [26]. As in the treatment-naive cohort, >10% had mutations at loci 20 (27%), 74 (13%) and 77 (15%). According to the genotypic resistance algorithm, those with mutations at point 74 (*n*=14 [13%]) had possible low-level resistance to nelfinavir (Table 4) [23]. Two (1.8%) individuals with mutations at point 33 had possible low-level resistance to fosamprenavir and tipranavir, and one (0.9%) individual had multiple protease inhibitor resistance because of mutations at positions 73 and 82 (Table 4).

Figure 2. Association between non-TAM resistance and development of TAMs in the reverse transcriptase gene



[a]Lamivudine (3TC) resistance, non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance or both. TAM, thymidine analogue mutation.

Discussion

With increasing access to ART in LMIC, increased numbers of patients are failing first-line therapy and thus being switched to second-line therapy [15]. In higher

income countries, both the choice of initial therapy and switch to second-line therapy are done with the use of individual genotypes [7]. In LMIC, a public health approach with more limited first- and second-line treatment options has been used. In this setting, it is crucial to understand the evolution of resistance patterns, and whether the current treatment regimens are adequate.

The majority of virus in this South African sample, in both treatment-naive and treatment-experienced patients, was clade C [24]. In both the treatment-naive and treatment-experienced group there were a number of mutations in the protease enzyme, possibly indicating divergence from clade B virus. The effect of these mutations on viral drug susceptibility is uncertain, but many, including L10I/V, K20R, M36I, L63P, A71V/T and V77I, are not expected to cause major drug resistance [28]. These mutations are similar to those noted in other African clade C virus and clade C consensus sequences [16–18,29]. Other mutations that are more likely to affect future use of a protease inhibitor, including D30N, M46I, I47V, I50V and V82A/F, were present, but in a minority of individuals, with only a single mutation noted per individual. The effect of such single mutations on the use of lopinavir in second-line therapy is not clear in our population.

There were very few mutations noted in the reverse transcriptase enzyme in the treatment-naive group. The two mutations that are likely to reduce susceptibility to NRTIs if transmitted, T215C and M41L, were not seen in either the treatment-naive or treatment-experienced groups [28]. The multiplicity of NNRTI mutations seen in the treatment-experienced group make NNRTI resistance the most likely to be transmitted in our population. Although the K103N mutation, which is expected to have the greatest effect on the use of NNRTIs, was not seen in the treatment-naive samples, there were two (1.6%) individuals each with a single mutation (Y181C and G190E) that would have some effect on NNRTI susceptibility. Although the currently recommended treatment regimens for first-line therapy remain appropriate, ongoing surveillance of NNRTI-resistant virus remains important [3,6].

The samples in the treatment-experienced group were taken from 110 individuals failing initial NNRTI therapy in the South African public sector. Previous data have shown that the rate of confirmed virological failure in this cohort was 5.6% at 32 months [15]. Adherence is monitored by tablet count in all public sector clinics in South Africa and any viral load increase should initiate a stepped-up adherence package including counsellor-driven re-education sessions, more regular clinic visits with emphasis on the use of a pill-box as a reminder system, as well as a home visit to assess living circumstances where the resources are available for this service [15]. Overall, 75% of those with an initial viral load breakthrough of >1,000 copies/ml again achieved suppression after structured adherence interventions [15]. For those in whom failure was confirmed with a second specimen >1,000 copies/ml, the median time from treatment commencement to noting initial virological breakthrough was 9 months.

The focus on adherence might explain the relatively small number of individuals with virological failure who did not have a significant drug-resistant mutation. Only seven (6.4%) individuals had wild-type virus at genotype, a smaller proportion than seen in the DART study (10%) [20]. Most of these individuals with confirmed failure had resistance mutations, which would exclude use of two of the antiretrovirals used in first-line regimens, that is, 3TC (83%; a similar proportion to that seen in the DART study of 70% [20], and the NNRTIs (86%). Resistance to both NNRTIs and 3TC (M184V) develops rapidly after initial virological breakthrough.

Resistance to the third drug in the regimen, the thymidine analogue, occurred more slowly. Although 23% of the group had ≥1 TAM, relatively few had two or three TAMs and the majority of individuals remained susceptible to both AZT and d4T. A trend towards TAM accumulation with prolonged time on failing therapy was noted, but was non-significant. People with lower $CD4^+$ T-cell counts at the time of genotyping were also more likely to have acquired a TAM, perhaps indicative of a longer time on failing therapy than noted here, because of the length of time between viral loads in this cohort. Thymidine analogues (AZT, ddI and lopinavir/ritonavir) are currently recycled in second-line therapy in South Africa, so TAM accumulation might reduce the efficacy of this therapy. However, if failure is identified before acquisition of TAMs, second-line therapy might remain more efficacious.

The increased presence (9%) of the K65R mutation in the treatment-experienced samples was unexpected, given the absence of abacavir or tenofovir in the South African treatment regimens. There is emerging evidence that non-subtype B virus might have a propensity to develop the K65R more readily compared with subtype B [10,25,26]. Doualla-Bell *et al.* [10] noted that d4T also selected for K65R in subtype C virus in Botswana, and that the mutation developed within 3 months of tenofovir therapy, unlike in subtype B where the K65R tends to emerge slowly in a small proportion of individuals on tenofovir. It is also possible that 3TC might select for the K65R mutation as recently described [22]. With the registration of tenofovir in South Africa in 2007, there is a push for the widespread use of this agent to replace d4T, initially in those experiencing adverse effects, but with the view to broad-spectrum first-line use. The likely rapid emergence of resistance to tenofovir in clade C virus should be of concern for treatment programmes, as the presence of this mutation

reduces susceptibility to all NRTIs except AZT, and thus would limit the choice of NRTIs for second-line therapy [30].

A limitation of this study is the 4–6 month window between viral load samples in the South African antiretroviral programme. Some individuals might have failed within weeks of their last suppressed viral load and others within days of their first raised viral load. Time from first virological breakthrough to time of genotype might therefore be an underestimate.

Resistance to the reverse transcriptase enzyme after exposure to NNRTI-containing first-line therapy follows a pattern that is predictable and similar to that of clade B: initial resistance to antiretrovirals that require a single point mutation, followed by slower development of resistance to drugs with a higher genetic barrier to resistance, such as the thymidine analogues.

Had second-line treatment been commenced within 6 months of initial virological breakthrough in the treatment-experienced group in this study, the likelihood of accumulating TAMs might have been reduced, with a potential increase in the efficacy of the recycled thymidine analogue in second-line therapy. Identification of and rapid response to virological failure is thus important to maintain the full benefit of second-line therapy. This would suggest clinical value to regular viral load testing to identify virological failure soon after it occurs, in contrast to a recently published model [31]. Because of the unexpected emergence of the K65R mutation in a substantial proportion of the cohort, tenofovir should be introduced cautiously with careful assessment of its effect on the emergence of resistance.

This study suggests that at present it is not crucial, in the context of the South African National ART programme, to have routine access to genotypes at baseline, as the vast majority of treatment-naive samples continue to be wild type. By contrast, the development of extensive resistance in those failing first-line therapy suggests that viral load monitoring is crucial and there could be a role for individual genotypes in those failing first-line therapy, particularly if second-line therapy is likely to be compromised by resistance to first-line therapy. Increased availability of low-cost assays for identifying resistance in patients in South Africa would be clinically valuable.

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Disclosure statement

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Additional File

The additional file 'Supplementary figures' can be accessed at www.intmedpress.com

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Quality of life and the impact of drug toxicities in a South African community-based antiretroviral programme

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Abstract

Background: The impact of highly active antiretroviral therapy (HAART) on health-related quality of life has been widely researched in the developed world, but there are few data from sub-Saharan Africa, where the vast majority of HIV-infected individuals live. This study examined health-related quality of life among HIV-positive individuals initiating HAART in Cape Town, South Africa, and explored the impact of HAART-related drug toxicities on quality of life.

Methods: Health-related quality of life was assessed using a standardised questionnaire, the Medical Outcomes Survey Short Form 36. Physical health summary scores and mental health summary scores were compared pre-HAART and at regular intervals during the first 48 weeks of HAART. The relationships between socio-demographic, baseline and on-treatment variables and decline in health-related quality of life, as well as the impact of drug toxicities on quality of life, were assessed in unadjusted bivariate and adjusted multivariate analyses.

Results: Two hundred and ninety-five patients were enrolled into the study. There was a significant increase in health-related quality of life during the first 48 weeks on HAART. The median physical health summary score increased from 45 to 53 units ($p < 0.001$) and median mental health summary score increased from 45 to 50 units ($p < 0.001$).

The bulk of this increase occurred during the first 16 weeks. Overall, 23% of participants experienced a decline in their physical health summary score, while 34% showed a decline in the mental health summary score. Average drops in median physical and mental health summary scores were 8.4 units (SD 9.31) and 9.9 (SD 11.4) units respectively. Participants with drug toxicity had lower physical health summary scores than participants without drug toxicity at all time points. However, only three participants with toxicity (27%) reported an actual decline in health-related quality of life by week 48. Drug toxicities had little impact on mental health summary scores.

Conclusion: These results confirm the health-related quality of life benefits of HAART. While the majority of patients experienced a significant improvement in health-related quality of life on HAART, up to a third of patients reported declines in this quality of life. This was largely related to better baseline clinical state. HAART-related drug toxicities did not have a significant impact on health-related quality of life during the first year of HAART, which supports the ongoing use of the current national first-line regimen.

Background

By December 2006, an estimated 39.5 million people worldwide were living with HIV and a further 2.9 million people had died due to AIDS. The bulk of infections (63% of the global burden) occurred in sub-Saharan Africa, where 24.7 million people were reported to be HIV infected [1]. In South Africa alone, 5.4 million people were estimated to be infected with HIV by the middle of 2006 and 600 000 were thought to have AIDS [2].

Prior to 2004, people infected with HIV in South Africa who were unable to access life-saving antiretroviral (ARV) therapy progressed to AIDS and died of their disease. The rollout of highly active antiretroviral therapy (HAART) through national and provincial programmes has dramatically altered this experience.

By late 2008, an estimated 549 700 HIV-positive individuals were receiving HAART in South Africa [3]. With increasing numbers of HIV-positive individuals being enrolled onto HAART and increasing survival among these patients, there is a growing need to understand the impact of HAART use on the quality of lives of HIV-infected individuals [4-8].

There is a sizeable body of research on the impact of HAART on health-related quality of life (HRQoL) in the developed world. Most recent cohort studies in the USA and Europe have shown no significant change in HRQoL within the first two years of HAART [9-11], although one study showed an increase in mental quality of life only [12], and two showed a decrease in physical quality of life [13,14].

Table 1: Demographic, baseline and on-treatment characteristics of female and male patients with any health-related quality of life data

Variable	Total	Female	Male	P-value
Number	295	219	76	-
Age (years) (mean, (SD)	34 (7)	33 (7)	38 (6)	<0.001
WHO stage 3 & 4 (n,(%))	254 (86)	184 (86)	70 (92)	0.079
Baseline CD4 count cells/mm^3 (median, (IQR))	88 (47; 148)	96 (52; 159)	77 (36; 130)	0.027
Baseline viral load copies/ml (median, (IQR))	80,876 (33,194; 201,784)	76,452 (31,547; 198,193)	87,763 (42,884; 211,938)	0.216
Baseline viral load log copies/ml (median, (IQR))	4.88 (4.52; 5.30)	4.88 (4.50; 5.30)	4.94 (4.63; 5.32)	0.216
Week 48 CD4 count cells/mm^3 (median, (IQR))	261 (183; 340)	265 (205; 365)	215 (171; 304)	0.004
Week 48 viral load copies/ml (median,(IQR))	49 (49; 49)	49 (49; 49)	49 (49; 49)	0.340
Week 48 viral load log copies/ml (median,(IQR))	1.69 (1.69; 1.69)	1.69 (1.69; 1.69)	1.69 (1.69; 1.69)	0.276
Change in CD4 count cells/mm^3 (mean, (SD))	178 (130)	184 (136)	159 (107)	0.152
Change in viral load log copies/ml (mean, (SD))	-2.96 (0.91)	-2.91 (0.95)	-3.11 (0.75)	0.107
Drug toxicity (n, (%))	11 (4%)	10 (5%)	1 (1%)	0.198

Table 2: Median scores for the eight health concepts pre-HAART and at week 16, 32 and 48 on HAART (n = 147)

	Pre-HAART	Week 16	Week 32	Week 48
Physical function	85	95	100	100
Physical role	50	100	100	100
Bodily pain	61	84	74	84
General health	54	77	77	72
Vitality	55	75	80	85
Social function	75	100	100	100
Emotional role	50	99	99	99
Mental health	68	72	76	72

In contrast, the 2NN study, which compared the efficacy and safety of three non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimens (nevirapine, efavirenz, and nevirapine plus efavirenz in combination with stavudine and lamivudine) showed an overall improvement in HRQoL over 48 weeks [15].

To date, only four studies have addressed the impact of HAART on HRQoL in developing countries [16-19]. Wouters et al [16] and Louwagie et al [17] assessed the impact of HAART on HRQoL in cross-sectional surveys, and showed a significant association between HAART and improved physical and emotional health. Unfortunately, the cross-sectional nature of these two studies and the limited time that participants were on HAART (six months or less) restrict the inferences that can be drawn from these studies.

Only the studies by Stangl et al [18] and Jelsma et al [19] assessed longitudinal changes in HRQoL associated with HAART use. Both reported a significant improvement in HRQoL over 12 months of HAART, with the bulk of this improvement occurring within the first three months on treatment.

Internationally, there is concern about the impact of HAART-related toxicity on HRQoL. In fact, it has been suggested that studies that consider only mortality outcomes ignore treatment-related morbidity and may actually overestimate the benefits of HAART [20].

These international concerns are echoed in South Africa and other developing countries, where national first-line regimens tend to be NNRTI-based and incorporate drugs such as stavudine, which has been shown to be the reason for up to 75% of drug switches for toxicity within the first three years of first-line HAART [21]. In South Africa, there is an ongoing debate about whether or not the side-effect profile of HAART may adversely affect the HRQoL of HIV-positive individuals.

More data about the impact of HAART and HAART-related toxicities on HRQoL are required in developing countries to inform programme and policy decisions about HAART roll-out strategies in order to maximise the quality of life of HIV-infected individuals.

Methods

Study population

This cohort study examined the HRQoL reported by HIV-positive individuals pre-HAART and at regular intervals during their first year of receiving HAART at the Hannan Crusaid Treatment Centre between September 2002 and March 2005. As per the national ARV guidelines, adult patients who had World Health Organization (WHO) Stage 4 disease and/or a CD4+ T cell count of <200 cells/mm^3 were commenced on first-line ARVs [22]. The majority of patients (99.6%) initiated on-treatment were ARV-naïve.

The Hannan Crusaid Treatment Centre is a community-based ARV clinic that was initiated in September 2002 as a joint venture between the Western Cape Department of Health, Desmond Tutu HIV Foundation and Crusaid, a UK-based non-governmental organization that raises funds to support people living with HIV/AIDS. The clinic was one of the first ARV rollout sites in the Western Cape Province of South Africa. It is situated alongside the primary community health care centre and boasts a multidisciplinary team of medical doctors, clinical nurse practitioners, clinic nurses, Sizophila adherence counsellors and a pharmacist.

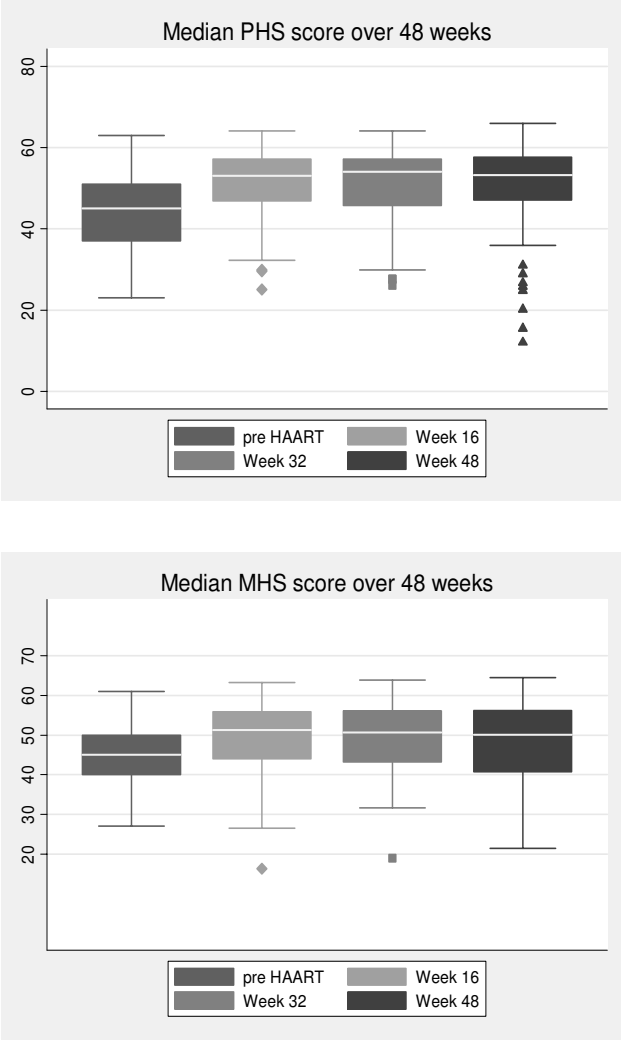

Figure 1
Change in median physical health summary score and median mental health summary score over the first 48 weeks of HAART.

The clinic followed a programmatic approach to ARV care with a standard first-line and second-line regimen. In keeping with WHO recommendations, the first-line regimen was NNRTI-based and the second was protease inhibitor-based [23].

Adults commenced on the first-line regimen (efavirenz or nevirapine plus stavudine and lamivudine) were reviewed at four, eight and 16 weeks, and thereafter every four months by a medical doctor. At these scheduled visits, patients were assessed clinically, virologically and immunologically. Patients who discontinued their first-line regimen – either due to virological failure or for toxicity reasons – were worked up for the second-line regimen (lopinavir/ritonavir, didanosine and zidovudine). Nucleoside reverse transcriptase inhibitor (NRTI) substitutions were made within regimen 1 or 2 for NRTI-associated toxicities.

Study procedures

At the screening visit, HIV-positive individuals met with the clinic nurse, who completed a demographic information sheet. Blood was drawn for viral load, CD4 cell count and safety blood testing (including a full blood count and liver function tests) at the screening visit and at all subsequent scheduled visits prior to the patient seeing the medical doctor.

The adherence counsellors were trained in the administration of the HRQoL instrument. The quality of life questionnaire was administered at each of the following scheduled visits: screening, baseline, week 16, week 32, week 48 and week 64. Although HRQoL data continued to be collected at scheduled visits following week 64, this study focused on quality of life only during the first year of ARVs.

HRQoL data were intended to be collected on all patients at all scheduled visits within the first year. This, however, was not always possible. Reasons for incomplete HRQoL data were: death, loss to follow up, transfer out, and patients leaving the clinic without the questionnaire being administered. The analysis only included those patients with HRQoL data available at all time points during the first year on HAART.

The University of Cape Town Research Ethics Committee approved all research activities involving antiretroviral service delivery and patient outcomes at the site. Patients signed a research consent form at the screening visit, indicating their willingness to take part in this research study.

Study measures

Quality of life

Health-related quality of life was assessed using a standardised questionnaire, the Medical Outcomes Survey Short Form 36 (MOS-SF36). The instrument uses 36 items to assess eight health concepts: (1) physical functioning; (2) role limitations because of physical health problems; (3) bodily pain; (4) social functioning; (5) general mental health; (6) role limitations because of emotional problems; (7) vitality; and (8) general health perceptions [24].

The MOS-SF36 questionnaire has been widely used in studies of quality of life in HIV-positive patients in both developed and developing countries, and has performed well in all of these settings [11,12,14,25-28]. The instrument has also undergone validity and reliability testing in a multiracial South African population and was able to differentiate between HIV-infected and non-infected individuals [28]. Population values exist for several countries,

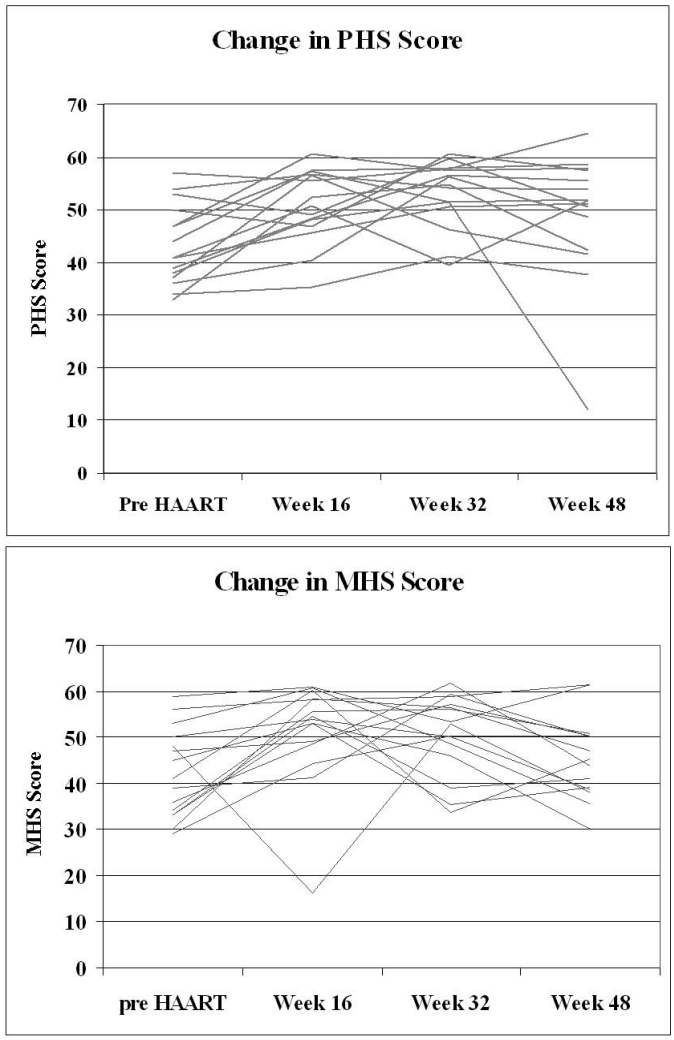

Figure 2
Change in physical health summary score and mental health summary score over the first 48 weeks of HAART for 15 random participants.

including South Africa. The English version of the instrument was used, with standard Xhosa explanations given by the counsellors for difficult concepts.

Quality of life data were entered using a custom-designed Epi Info™ template to ensure high data quality. On completion of data entry for each questionnaire, scores for the eight health components were automatically generated according to standard scoring algorithms. Data were then transferred into a Microsoft Excel spreadsheet where health component scores were transformed into the physical health summary (PHS) and mental health summary (MHS) scores using standardised factor analysis-based weights.

Missing questionnaire items were estimated using a standard scoring algorithm that estimates missing values [24]. Scores for screening and baseline were combined to form an average pre-HAART score. For those participants whose week 48 scores were not available, week 64 scores were used to replace missing data. This replacement of scores was deemed acceptable as data analysis demonstrated no significant difference between overall week 48 and week 64 scores.

Socio-demographic and clinical information

Demographic information was collected using standard paperwork. Patients were staged according to WHO clinical criteria by the medical doctor at their screening visit. The Toga Laboratory performed viral load and CD4 cell count testing. Viral load testing made use of the branch DNA hybridisation technique (Versant™ HIV-1 RNA 3.0 branched chain DNA assay, Bayer HealthCare, Leverkusen, Germany) and CD4+ T cell counts were measured by flow cytometry (FACSCount™, Becton Dickinson, Franklin Lakes, NJ, USA).

Table 3: Factors associated with negative physical health summary score at week 48

Variable		Univariate model	Multivariate model 1	Multivariate model 2
		Odds ratio, 95% CI (P-value)	Odds ratio, 95% CI (P-value)	Odds ratio, 95% CI (P-value)
Age (continuous)		1.04, (0.98; 1.09) 0.184	1.08, (1.01; 1.15) 0.034	1.07 (1.01; 1.14) 0.029
Age (≤ 34 = 0, >34 = 1)		1.20, (0.56; 2.60) 0.637		
Gender (male = 0, female = 1)		0.55, (0.19; 1.56) 0.260	0.52, (0.14; 1.99) 0.341	
WHO stage (1&2 = 0, 3&4 = 1)		0.38, (0.13; 1.08) 0.068	0.61, (0.17; 2.15) 0.441	
Baseline CD4 count cells/mm^3 (>50 = 0, ≤ 50 = 1)		0.42, (0.15; 1.17) 0.098	0.93, (0.25; 3.41) 0.910	
Baseline viral load log copies/ml (≤ 5 = 0, >5 = 1)		0.24, (0.10; 0.59) 0.002	0.48, (0.14; 1.65) 0.245	0.22, (0.08; 0.60) 0.003
Pre-HAART PHS score (continuous)		1.14 (1.07; 1.21) <0.001	1.16 (1.08; 1.24) <0.001	1.15 (1.08; 1.23) <0.001
Week 48 CD4 count cells/mm^3 (<250 = 0, ≥ 250 = 1)		0.63, (0.29; 1.37) 0.245		
Week 48 CD4 count cells/mm^3	<200	1		
	200–350	0.62, (0.25; 1.51) 0.29		
	350–500	0.67, (0.22; 2.07) 0.483		
	≥ 500	0.42, (0.08; 2.21) 0.309		
Week 48 viral load copies/ml (<50 = 0, ≥ 50 = 1)		0.96, (0.37; 2.48) 0.935		
Week 48 viral load copies/ml	<50	1		
	50–399	0.39, (0.08; 1.79) 0.224		
	400–4999	1.10, (0.11; 11.00) 0.936		
	≥ 5000	3.30, (0.77; 14.07) 0.107		
Week 48 log viral load log copies/ml (<1.69 = 0, ≥ 1.69 = 1)		0.96, (0.37; 2.48) 0.935		

Table 3: Factors associated with negative physical health summary score at week 48 *(Continued)*

Week 48 log viral load log copies/ml	<1.69	1	
	1.69–2.59	0.58, (0.16; 2.14) 0.414	
	2.60–3.69	1.10, (0.11; 11.00) 0.936	
	≥ 3.69	2.47, (0.51; 11.74) 0.225	
Δ CD4 count cells/mm^3		0.995, (0.991; 0.999) 0.010	0.998, (0.993; 1.003) 0.420
Δ log viral load log copies/ml		0.58, (0.37; 0.90) 0.015	0.66, (0.36; 1.27) 0.209

Drug toxicities were detected by the medical doctor at both scheduled and unscheduled clinical visits through clinical questioning, examination and safety blood draws (including a full blood count, liver function tests, amylase and lactate levels as requested). Drug toxicities were defined as any adverse event thought by the clinician to be HAART-related and that required a change in antiretroviral therapy. Drug changes could either be a NRTI substitution or a change from NNRTI to a protease inhibitor.

Statistical analysis

The cohort was initially described using means, medians and proportions, as appropriate. Changes in HRQoL pre-HAART and at week 16, 32 and 48 were compared using the Wilcoxon Rank Sum Test. Crude associations were first examined using Fisher's Exact, Chi Squared and Wilcoxon Rank Sum tests, as appropriate.

Negative HRQoL was defined as a decrease in PHS or MHS scores between pre-HAART and week 48. Univariate relationships were then explored between the outcome variables – negative PHS and negative MHS – and each explanatory variable. Multivariate analyses made use of logistic regression models to examine the adjusted association between negative HRQoL and various socio-demographic, baseline and on-treatment explanatory variables as appropriate.

Multivariate analysis started with a full model (Model 1) and explanatory variables were removed in a stepwise manner until the final model (Model 2) was selected. All final logistic regression models were checked against model assumptions. Outliers and potentially influential observations were identified and examined to ensure that model results were not being unduly influenced by a small number of non-representative observations. Models were rerun with selected observations excluded.

All statistical analyses were performed using Intercooled Stata Version 8.2 (Stata Corporation, College Station, Texas, USA). All statistical tests are two-sided at alpha = 0.05.

Results

Of the 295 patients with any HRQoL data, 292 (99%) had baseline data, 271 (92%) had week 16 data, 233 (79%) had week 32 data, and 179 (61%) had week 48 data. Complete HRQoL data, obtained pre-treatment and at every scheduled on-treatment visit, were available for 147 patients.

Demographic, baseline and on-treatment characteristic

Table 1 describes the demographic and baseline characteristics of the 295 patients with any HRQoL data. The average age of the cohort was 34 years (standard deviation: 4) and 74% of patients were female (n = 219). Eighty-six percent of patients (n = 370) had WHO Stage 3 and 4 disease.

The median baseline CD4 count was 88 cells/mm^3 (inter quartile range: 44, 154) and median baseline log viral load was 4.9 (inter quartile range: 4.5, 5.3). Men were older and had more advanced disease than women. The majority of drug toxicities (90.9%) occurred in women with only one drug change made due to toxicity among men. There were no differences in demographic and baseline characteristics between patients with complete HRQoL data (n = 147) and those with incomplete data (n = 148).

Health-related quality of life data

The median scores for the eight health concepts pre-treatment and at regular intervals on-treatment are described in Table 2. The scores all demonstrated an increase in HRQoL between pre-HAART and week 48, with the greatest increase occurring at week 16 ($p < 0.001$).

Table 4: Factors associated with negative mental health summary score at week 48

Variable		Univariate model	Multivariate model 1	Multivariate model 2
		Odds ratio, 95% CI (P-value)	**Odds ratio, 95% CI (P-value)**	**Odds ratio, 95% CI (P-value)**
Age (continuous)		1.01, (0.96; 1.06) 0.767		
Age (<34 = 0, >34 = 1)		1.49, (0.75; 2.96) 0.258	2.20, (0.94; 5.15) 0.068	1.77, (0.83; 3.78) 0.142
Gender (male = 0, female = 1)		0.58, (0.24; 1.41) 0.227	0.65, (0.23; 1.85) 0.419	
WHO stage (1&2 = 0, 3&4 = 1)		0.94, (0.33; 2.71) 0.906	1.45, (0.44; 4.83) 0.543	
Baseline CD4 count cells/mm^3 (>50 = 0, <50 = 1)		0.35, (0.14; 0.86) 0.022	0.38, (0.14; 1.07) 0.067	0.41, (0.16; 1.09) 0.075
Baseline viral load log copies/ml (<5 = 0, >5 = 1)		0.41, (0.20; 0.83) 0.014	0.55, (0.20; 1.49) 0.239	0.50, (0.23; 1.09) 0.081
Pre-HAART MHS score (continuous)		1.10 (1.05; 1.16) <0.001	1.10 (1.04; 1.16) <0.001	1.09 (1.04; 1.15) 0.001
Week 48 CD4 count cells/mm^3 (<250 = 0, >250 = 1)		1.67, (0.83; 3.37) 0.151		
Week 48 CD4 count cells/mm^3	<200	1		
	200–350	5.18, (1.92; 13.96) 0.001		
	350–500	2.39, (0.72; 7.91) 0.155		
	>500	2.52, (0.58; 10.88) 0.216		
Week 48 viral load copies/ml (<50 = 0, >50 = 1)		1.30, (0.57; 2.94) 0.535		
Week 48 viral load copies/ml	<50	1		
	50–399	0.95, (0.33; 2.69) 0.919		
	400–4999	2.05, (0.28; 15.14) 0.481		
	>5000	2.05, (0.49; 8.66) 0.327		
Week 48 viral load log copies/ml (<1.69 = 0, >1.69 = 1)		1.30, (0.57; 2.94) 0.535		

Table 4: Factors associated with negative mental health summary score at week 48 *(Continued)*

Week 48 viral load log copies/ml	<1.69	1	
	1.69–2.59	0.88, (0.31; 2.47) 0.808	
	2.60–3.69	2.05, (0.28; 15.14) 0.481	
	>3.69	2.74, (0.58; 12.85) 0.202	
Δ CD4 count cells/mm^3		0.999, (0.996; 1.002) 0.414	1.002, (0.998; 1.01) 0.389
Δ log viral load log copies/ml		0.67, (0.44; 1.01) 0.054	0.74, (0.43; 1.28) 0.282

The physical health summary and mental health summary scores also showed an improvement in HRQoL over time (Figure 1). There was a significant increase in both summary scores between pre-HAART and week 16. The median PHS score increased from 45 to 53 units ($p < 0.001$) and the median MHS score increased from 45 to 51 units ($p < 0.001$). These increases were then maintained through weeks 32 and 48.

However, not all participants experienced a linear increase in HRQoL. Using a random sample of 15 participants, it was evident that while the bulk of participants experienced a gradual improvement in HRQoL, others experienced a worsening of HRQoL (Figure 2). While the average change in PHS score between pre-HAART and week 48 was an increase of seven units (standard deviation: 11.9), 23% of participants experienced a decrease in PHS score during this period. The average drop in PHS score among these participants was 8.4 units (standard deviation: 9.31).

Similarly, while MHS score increased by an average of 3.3 units (standard deviation: 11.4) between pre-HAART and week 48, 34% of participants experienced a decline in MHS score. The average drop in MHS score among these participants was 9.9 units (standard deviation: 5.92).

Factors associated with negative change in quality of life

Baseline log viral load, pre-HAART PHS score, change in CD4 count and change in log viral load were all strongly associated with a negative PHS in the univariate analyses (Table 3).

In the multivariate regression model, pre-HAART PHS score and baseline log viral load were the strongest predictors of negative PHS at week 48. Participants with a higher pre-HAART HRQoL score were more likely to report negative PHS (OR1.15; 95% CI 1.08, 1.23; $p < 0.001$), whereas participants with a higher baseline log viral load (>5.0 log) were less likely to report negative PHS than participants with a lower baseline log viral load (≤ 5.0 log) (OR 0.22; 95% CI 0.08, 0.60; $p = 0.003$).

Age was also predictive of negative PHS. Older participants (above 34 years of age) were more likely to report negative PHS than younger participants (OR 1.07; 95% CI 1.01, 1.14, 0.085; $p = 0.029$). Neither gender nor any of the week 48 variables were associated with negative PHS.

Baseline CD4 count, baseline log viral load, pre-HAART MHS score and change in log viral load were all associated with negative MHS in the univariate models (Table 4). In the multivariate regression model, pre-HAART MHS score was the strongest predictor of negative MHS at 48 weeks. Participants with higher pre-HAART HRQoL scores were more likely to report negative MHS than participants with lower pre-HAART MHS scores (OR 1.09; 95% CI 1.04, 1.15; $p = 0.001$).

Baseline CD4 count and baseline log viral load remained weakly associated with the outcome. Participants with lower baseline CD4 count (≤ 50 cells/mm^3) were less likely to experience negative MHS than participants with higher baseline CD4 count (>50 cells/mm^3) (OR 0.41; 95% CI 0.16, 1.09; $p = 0.075$). Participants with a higher baseline log viral load (>5.0 log) were less likely to experience negative MHS than participants with lower baseline log viral load (≤ 5.0 log) (OR 0.50; 95% CI 0.23, 1.09; $p = 0.081$). Gender and the week 48 variables were not predictive of negative MHS at 48 weeks.

Drug toxicities and quality of life

Eleven participants experienced drug-related toxicities during the first 48 weeks of HAART. Ninety-one percent (n = 10) of these toxicities occurred in women, with 50% (n = 5) of these being due to lactic acidosis. Participants experiencing drug toxicities had similar demographic and baseline characteristics to the overall cohort.

Table 5: Description of drug toxicities occurring during the first year of HAART

Description	Week 0–16	Week 16–32	Week 32–48	Total
Any toxicity	2	2	7	11
EFV hypersensitivity reaction	2	***	1	3
Peripheral neuropathy	***	2	***	2
Elevated transaminases	***	***	1	1
Hyperlactataemia/lactic acidosis	***	***	5	5

Table 5 describes the types of drug toxicities that occurred during the first year of HAART. Efavirenz hypersensitivity reactions were the cause of drug toxicities within the first 16 weeks of HAART. Between weeks 16 and 32, peripheral neuropathies were the main reason for drug changes. Elevated transaminases and hyperlactatemia were the main causes of drug toxicities between weeks 32 and 48 of HAART.

One patient experienced an efavirenz hypersensitivity reaction between weeks 32 and 48. This was due to the fact that the patient was switched to efavirenz at this time. The bulk (64%) of toxicities occurred during the week 32 to 48 interval and were mostly elevated transaminases and hyperlactataemia related to stavudine use.

The 11 participants who experienced drug toxicity during the first 48 weeks of HAART achieved lower PHS scores at all time points than the 281 participants who did not have toxicity. While these differences were not statistically significant pre-HAART and at weeks 16 and 32, they did become significant at week 48. The median PHS score at week 48 was 50 for participants with drug toxicity, compared to 53 for participants without drug toxicity ($p = 0.0053$) (Table 6).

Drug toxicities did not appear to have a significant impact on median MHS scores over the first 48 weeks of HAART. The 11 participants with drug toxicity had a lower median MHS score pre-HAART than the 281 participants without drug toxicity, but this difference was not statistically significant (42 versus 45, $p = 0.1793$). There was no impact of baseline mental health status on the reporting of toxicities. At weeks 16, 32 and 48, participants with drug toxicity reported higher median MHS scores than those without toxicity. Again, these differences were not statistically significant (Table 6).

Examining the associations between drug toxicity and negative HRQoL, it was noted that only three (27%) of all drug toxicities occurred among participants who reported negative PHS and that these toxicities occurred during the week 32 to 48 treatment interval. No drug toxicities occurred among participants who reported negative MHS.

Discussion

This study reported a significant increase in HRQoL during the first 48 weeks on HAART, with the bulk of this increase occurring during the first 16 weeks on treatment. Improvement in HRQoL occurred across all core domains assessed, as well as the physical health summary and mental health summary.

This study therefore supports the findings of Stangl et al [18] and Jelsma et al [19] who both reported an increase in HRQoL within the first three months of therapy in similar patient populations. The dramatic increase in HRQoL during the first few weeks of HAART occurred over the time period when patients usually experience the most significant gains in health. The greatest decrease in viral load happens within the first few weeks of treatment and mortality and morbidity rates begin to fall after just a month on HAART [29,30].

There have been few analyses dealing with declines in PHS and MHS scores. In fact, negative HRQoL is often overshadowed by the overwhelming positive impact of HAART on HRQoL, and is therefore not reported. This research showed that although there was a general improvement in HRQoL on HAART, up to a third of participants experienced a decline in HRQoL during the first 48 weeks of HAART. Twenty-three percent of participants reported a drop in PHS score and 34% reported a drop in MHS score.

The most significant predictors of negative PHS and MHS were baseline HRQoL score, baseline log viral load and baseline CD4 count. The association between higher baseline HRQoL score and negative HRQoL could have been due to the fact that patients with higher baseline scores had less room for improvement and were therefore more likely to regress to the mean.

Table 6: Median physical health summary and median mental health summary scores and drug toxicity

	N	Pre-HAART	Week 16	Week 32	Week 48
Median PHS					
Drug toxicity	11	39	51	52	50
No drug toxicity	281	44	52	53	53
p-value		0.854	0.225	0.390	0.005
Median MHS					
Drug toxicity	11	42	51	51	52
No drug toxicity	281	45	50	49	48
p-value		0.179	0.539	0.326	0.912

Median PHS and median MHS pre-HAART and at week 16, 32 and 48 in patients with any drug toxicity at any time in the first year compared to patients without drug toxicity.

Baseline log viral load was strongly associated with negative PHS. Participants with higher baseline log viral loads were less likely to report negative PHS than participants with lower baseline log viral loads. Similarly, baseline log viral load and baseline CD4 count were associated with negative MHS. Participants with higher baseline log viral loads or lower baseline CD4 counts were less likely to report negative MHS than participants with lower baseline log viral loads or higher baseline CD4 counts.

Participants with more advanced disease, characterised by higher baseline viral loads and lower baseline CD4 counts, were less likely to report a decline in HRQoL than those with earlier disease. So it was the relatively well patients entering into the programme who were at greatest risk of experiencing negative HRQoL. These associations could be explained by the negative impact of symptoms on HRQoL [10-12]. Patients with more advanced disease are more likely to have a greater number or intensity of symptoms than patients with less advanced disease pre-HAART [10], but once on HAART, these symptoms improve [11,12].

Although there is great concern about the impact of drug toxicities on HRQoL, few studies have directly assessed this association. In the developed world, symptoms have been shown to impact negatively on both physical and mental HRQoL [10,31]. However, the nature of the symptoms and whether they were attributed to the disease process or to HAART was not clear.

In the developing world, Jelsma et al [19] concluded that possible side effects of HAART had a negligible impact on HRQoL. This conclusion was based on the overall increase in HRQoL demonstrated for the cohort and did not specifically address those patients who experienced a decline in HRQoL.

Few drug toxicities were recorded during the first 48 weeks on HAART. While participants who experienced drug toxicity had lower PHS scores than participants without a drug toxicity at all time points (most notably during the 32 to 48 week treatment interval), only three (27%) participants with toxicity reported an actual decline in physical HRQoL between pre-HAART and week 48.

Drug toxicities, especially those related to stavudine use, may have a negative impact on physical HRQoL at the time of the toxicity. They do not, however, reduce overall gains in HRQoL. Drug toxicities had little impact on mental HRQoL.

The greatest strength of this study is its longitudinal design. This allowed the assessment of the associations between various socio-demographic and clinical factors and HRQoL, and allowed for inferences to be made about causal relationships.

Limitations to the study included possible selection bias and information bias related to the use of the MOS-SF36 instrument. The loss of up to 50% of patients, who had incomplete HRQoL data, from the final analysis may have resulted in a selection bias towards the healthier section of the cohort. However, as there were no significant differences in demographic, baseline and on-treatment characteristics between patients with incomplete and complete

HRQoL data, it is unlikely that this was the case. This did impact on the number of patients available for analysis though, and may have limited the ability of the study to detect significant associations.

The MOS-SF36 instrument is a generic HRQoL measure and, like all generic instruments, may not be sensitive enough to measure the more specific aspects of HRQoL impacted on by the HIV disease process. This could lead to either an underestimation or overestimation of HRQoL scores and, more important, to a change in HRQoL scores, thereby under-reporting or over-reporting the actual impact of HAART on HRQoL.

The MOS-SF36 instrument is also prone to ceiling effects, where substantial numbers of patients get the highest possible score for a domain. This would have made it difficult for the instrument to pick up changes in HRQoL at the upper end of the scale, and may have been a problem with increasing time on treatment. Ceiling effects could have lead to the underestimation of HRQoL gains.

This study reported on HRQoL during the first 48 weeks of HAART only. As HAART-related drug toxicities, especially those related to stavudine, increase with length of time on HAART [21], this study may have under-reported the impact of drug toxicities on gains in HRQoL.

Furthermore, this study only considered drug toxicities that were severe enough to prompt a change in antiretroviral therapy. Less severe toxicities that may also have impacted negatively on HRQoL were not reported on. This could have led to an underestimation of the true negative impact of drug toxicities on HRQoL.

Conclusion

This study confirmed the overwhelmingly positive HRQoL benefits of HAART in a community ARV clinic in South Africa. HRQoL improved significantly during the first 48 weeks of HAART, with the bulk of improvement occurring during the first 16 weeks of treatment.

Up to a third of patients experienced a decline in HRQoL on HAART. This was largely related to the patient's baseline clinical state. HAART-related drug toxicities did not have a significant impact on HRQoL during the first year of HAART, supporting the ongoing use of the current national first-line regimen.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JP participated in the design of the study and its coordination, acquired the data, performed the statistical analysis, interpreted the data, and drafted the manuscript. LM assisted with the statistical analysis and interpretation of the data, and helped to draft the manuscript. RW conceived of the study, participated in its design, and critically reviewed the manuscript. All authors have read and approved the final manuscript.

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Nurse versus doctor management of HIV-infected patients receiving antiretroviral therapy (CIPRA-SA): a randomised non-inferiority trial

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Summary

Background Expanded access to combination antiretroviral therapy (ART) in resource-poor settings is dependent on task shifting from doctors to other health-care providers. We compared outcomes of nurse versus doctor management of ART care for HIV-infected patients.

Methods This randomised non-inferiority trial was undertaken at two South African primary-care clinics. HIV-positive individuals with a CD4 cell count of less than 350 cells per µL or WHO stage 3 or 4 disease were randomly assigned to nurse-monitored or doctor-monitored ART care. Patients were randomly assigned by stratified permuted block randomisation, and neither the patients nor those analysing the data were masked to assignment. The primary objective was a composite endpoint of treatment-limiting events, incorporating mortality, viral failure, treatment-limiting toxic effects, and adherence to visit schedule. Analysis was by intention to treat. Non-inferiority of the nurse versus doctor group for cumulative treatment failure was prespecified as an upper 95% CI for the hazard ratio that was less than 1·40. This study is registered with ClinicalTrials.gov, number NCT00255840.

Findings 408 patients were assigned to doctor-monitored ART care and 404 to nurse-monitored ART care; all participants were analysed. 371 (46%) patients reached an endpoint of treatment failure: 192 (48%) in the nurse group and 179 (44%) in the doctor group. The hazard ratio for composite failure was 1·09 (95% CI 0·89–1·33), which was within the limits for non-inferiority. After a median follow-up of 120 weeks (IQR 60–144), deaths (ten *vs* 11), virological failures (44 *vs* 39), toxicity failures (68 *vs* 66), and programme losses (70 *vs* 63) were similar in nurse and doctor groups, respectively.

Interpretation Nurse-monitored ART is non-inferior to doctor-monitored therapy. Findings from this study lend support to task shifting to appropriately trained nurses for monitoring of ART.

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Introduction

Combination drug therapy has had a remarkable effect on the reduction of AIDS-related morbidity and mortality.[1] In industrialised countries, antiretroviral management is administered by specialist physicians who prescribe from the full range of available antiretroviral drugs, supported by frequent laboratory monitoring including resistance testing.[2] Finding from several studies in industrialised settings have shown that outpatients have better outcomes when cared for by a physician with HIV expertise than do those without such a physician, including quality of care and survival,[3–7] which could be an indicator of the complexities of HIV infection and its management.[2] By contrast with the small epidemic in resource-rich countries, there are 22·4 million people living with HIV in sub-Saharan Africa,[8] with an estimated 3·8 million in urgent need of treatment.[9] Globally, there is a shortage of 4·3 million health workers (doctors, midwives, nurses, and support workers);[9] in South Africa there are only 17·4 medical practitioners per 100 000 people, who are largely concentrated in urban areas.[10,11]

By contrast with the individualised approach to HIV care in developed countries, WHO has proposed a public health approach to antiretroviral therapy (ART) to enable scaling up of access to treatment for large numbers of HIV-positive adults and children in developing countries.[12] An approach using standardised simplified treatment protocols and decentralised service delivery was developed to enable lower level health-care workers to deliver care.[13] Models of care have investigated task shifting to clinical officers[14] and a combination of nurses and community workers;[15] however, nurse-led models of antiretroviral delivery have been one of the most widely implemented models of HIV care in poor-resourced African settings.[15–18] Findings from a trial have shown that work-site treatment of hypertension by specially trained nurses led to significantly improved blood pressure control and drug adherence.[19] So far no randomised prospective study has been published to show the effectiveness of nurse-

monitored ART. The HIV/AIDS strategic plan of South Africa, a middle-income country with the world's largest national ART programme, envisions increasing reliance on nurses for monitoring of ART.[20] With increasing deployment of nurses for HIV care, operational research is urgently needed to establish whether nurse-led models of care are safe and effective.

We therefore compared outcomes of nurse versus doctor management of doctor-initiated ART care for HIV-infected patients.

Methods

Study design and population

This prospective, unblinded, randomised controlled study was a community-based ART strategy trial done as part of the Comprehensive International Program for Research in AIDS in South Africa (CIPRA-SA).[21] The trial was undertaken between Feb 25, 2005, and Jan 20, 2009, at two primary health-care sites in South African townships: Masiphumelele in Cape Town and Soweto in Johannesburg. The CIPRA-SA study compared two treatment monitoring strategies. Participants were allocated to receive either their primary care from doctors (hereafter referred to as doctor group) or from primary health-care nurses (hereafter referred to as nurse group). The standard-care strategy (doctor group) was consistent with the routine management of patients in the current South African ART programme, which is based on treatment initiated and monitored by a doctor.[13] The experimental nurse monitoring strategy used doctor-initiated ART monitored by primary health-care nurses. To ensure that all procedures and overall study management conformed to the national guidelines,[22] and National Institutes of Health guidelines for research on human patients,[23] a clinical safety team was established consisting of clinicians with experience of research.

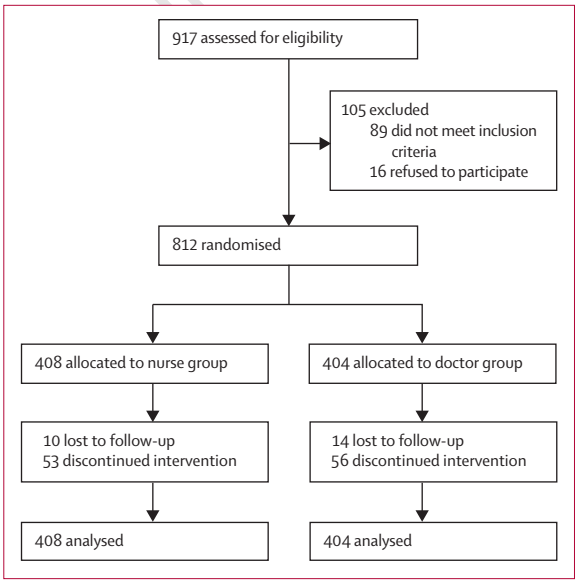

Figure 1: **Trial profile**

The eligible study population consisted of adults with HIV-1 infection who had received less than 6 weeks of ART, aged older than 16 years, with a CD4 cell count less than 350 cells per µL or a previous AIDS-defining illness,[24] and not in the first trimester of pregnancy. Women who had received short-course ART for prevention of mother-to-child transmission were not excluded. Screening laboratory investigations for renal function, liver enzymes, and haematology had to be less than grade 3 by the National Institutes of Health Division of AIDS toxicity grading scale.[25] An active opportunistic infection was exclusionary if the patient's treatment status was not considered stable (ie, treatment for <7 days) or in the case of tuberculosis if treatment had been for less than 8 weeks (amended in October, 2005, to <2 weeks of tuberculosis treatment). Other exclusion criteria were concomitant treatment with systemic myelosuppressive, neurotoxic, pancreatotoxic, hepatotoxic, or cytotoxic treatment within 30 days of randomisation; acute hepatitis; intractable diarrhoea (lasting >6 weeks); bilateral peripheral neuropathy of grade 2 or higher; and drug or alcohol misuse that was considered by the investigator to potentially interfere with study compliance.

The study was approved at the institutional review boards of the University of Cape Town and University of the Witwatersrand, and written informed consent was obtained from all participants before the start of study procedures.

Randomisation and masking

The clinical safety team was responsible for the recruitment of participants including consent, screening processes, start of therapy, and provision of ongoing consultation support by telephone to study nurses and doctors. Participants were randomly assigned in a ratio of 1:1 within sites. Randomisation lists were generated centrally with a stratified permuted block randomisation (with blocks of six). The strata corresponded to the different study sites. The allocation codes for a particular site were sealed in sequentially numbered envelopes, reflecting their order on the randomisation list, and distributed to the site. At randomisation, the site pharmacist unsealed the sequential envelope to reveal the randomisation code and participant randomisation number. Neither the participant nor those analysing the data were masked to the assignment.

Procedures

At each site the experimental group (nurse) used two experienced primary health-care nurses. Primary health-care nurses are a nationally registered cadre of nurses who have undergone 1 additional year of clinical training in primary health care. The control group (doctor) consisted of two doctors (medical officers with no previous HIV-care experience) at each site. Primary-care providers who had little or no previous experience with ART were selected for both groups of the study. Each new

clinician (nurse or doctor) received similar structured didactic and clinical training in HIV and the use of ART from the clinical safety team. To limit contamination between randomised groups, work activity and monitoring schedules were separated with routine visits scheduled on different days of the week, although at least one clinician was available to undertake unscheduled visits in the other group of the study. Whenever possible the participant saw the same clinician within the treatment group. Both groups were supported by a clinic nurse who ensured that the participant saw the correct clinician, did routine clinic procedures, and scheduled further patient visits; and by a team of lay community counsellors who were trained in treatment adherence counselling. A pharmacist oversaw ordering and dispensing of antiretroviral drugs at each site.

The primary study outcome was a composite endpoint of possible treatment-limiting events that could occur on first-line ART. The composite endpoint was chosen to represent both biological measures of treatment safety, efficacy, and disease progression, and measures of patient dissatisfaction with the treatment strategy. These outcomes were: all-cause mortality, loss to follow-up, virological failure, toxicity failure, withdrawn consent, defaulting clinic schedule, and HIV-disease progression. Virological failure was defined as either a decline of less than 1·5 $\log_{10}$ in viral load from baseline to 12 weeks of treatment (early failure), or two consecutive viral loads 4 weeks apart of more than 1000 copies per mL (late failure).

Toxicity failure was defined as grades 3 and 4 adverse events or other events needing treatment interruption for more than 42 days.[25] However, single-drug substitution as a result of drug-related toxic effects was not regarded as failure if treatment was interrupted for less than 42 days.

Patients who missed three consecutive study visits and were not able to be contacted by the study team were defined as lost to follow-up. Defaulting clinic schedule was defined as missing three or more consecutive scheduled clinic appointments with a study visit window of 7 days, but patients were able to be traced. Disease progression was defined by new AIDS-defining clinical events, as classified in the revised case definition of the Centers for Disease Control and Prevention (CDC).[24] Tuberculosis is hyperendemic in South Africa and therefore pulmonary tuberculosis was not included in the composite endpoint but was analysed separately.

Throughout the study, a data monitoring team reviewed data from all study visits to identify any default or loss to follow-up. An endpoint review committee reviewed all events classified as death and toxicity failure to ascertain whether the correct assignment to study regimen and procedure were undertaken. An independent data and safety monitoring board reviewed the safety and efficacy of the CIPRA-SA study every 6 months.

ART was provided and specified by the South African Department of Health. Regimens initially prescribed by the clinical safety team[26] included a nucleoside backbone of stavudine and lamivudine, with a choice of efavirenz, nevirapine, or lopinavir plus ritonavir. The initial dose of stavudine was 40 mg daily for individuals weighing more than 60 kg, which was reduced to 30 mg for all patients from mid-2007 in line with WHO recommendations.[27] Efavirenz was the preferred non-nucleoside for men and women not wishing to become pregnant and willing to maintain both barrier and hormonal contraception throughout the study. Women of childbearing potential were prescribed nevirapine if their CD4+ lymphocyte count was less than 250 cells per µL, or lopinavir plus ritonavir if their count was 250 cells per µL or greater. Pregnant women, who were allowed to enrol after their first trimester, were prescribed either nelfinavir or lopinavir plus ritonavir.

After consenting and randomisation by the clinical safety team, the primary-care provider of the assigned group undertook responsibility for treatment initiation, adherence counselling, and follow-up visits. Patients were scheduled for study visits at baseline and then at weeks 2,

	Nurse group (N=404)	Doctor group (N=408)
Women	297 (73·5%)	276 (67·7%)
Age (years)	32·3 (28·0–36·6)	32·2 (28·0–37·4)
BMI (kg/m²)	23·5 (21·3–27·6)	23·5 (20·4–26·8)
CDC classification		
Class A	160 (39·6%)	141 (34·6%)
Class B	111 (27·5%)	118 (28·9%)
Class C	133 (32·9%)	149 (36·5%)
CD4 cell count (cells per µL)		
<200	260 (64·4%)	257 (63·0%)
200–350	119 (29·5%)	131 (32·1%)
>350–500	23 (5·7%)	18 (4·4%)
>500	2 (0·5%)	2 (0·5%)
Median (IQR)	165 (105–235)	164 (110–225)
Viral load (copies per mL)		
≤100 000	181 (44·8%)	170 (41·7%)
>100 000	223 (55·2%)	238 (58·3%)
$\log_{10}$ viral load	4·99 (0·75)	5·09 (0·73)
Baseline regimen prescribed		
Stavudine, lamivudine, efavirenz	293 (72·5%)	304 (74·5%)
Stavudine, lamivudine, nevirapine	72 (17·8%)	81 (19·9%)
Stavudine, lamivudine, lopinavir/ ritonavir*	35 (8·7%)	20 (4·9%)
Stavudine, lamivudine, nelfinavir*	4 (1%)	3 (0·7%)
Previous exposure to antiretrovirals†		
Single-dose nevirapine	81 (20%)	86 (21·1%)
Zidovudine	2 (0·5%)	4 (1·0%)
Nevirapine, zidovudine	14 (3·5%)	15 (3·7%)
Triple-drug therapy	1 (0·2%)	0 (0%)

Data are number (%), median (IQR), or mean (SD). BMI=body-mass index. CDC=Centers for Disease Control and Prevention. *Regimens containing protease inhibitors were prescribed to pregnant women or women of childbearing potential with CD4 count greater than 250 cells per µL who were unable or unwilling to use both a barrier contraceptive and a progesterone contraceptive. These women could not receive a regimen containing either nevirapine or efavirenz. †Previous exposure to antiretroviral therapy for prevention of transmission, either from mother-to-child or in postsexual exposure. Prophylaxis was allowed by the protocol for up to 6 weeks of treatment.

Table 1: **Baseline demographic and clinical characteristics**

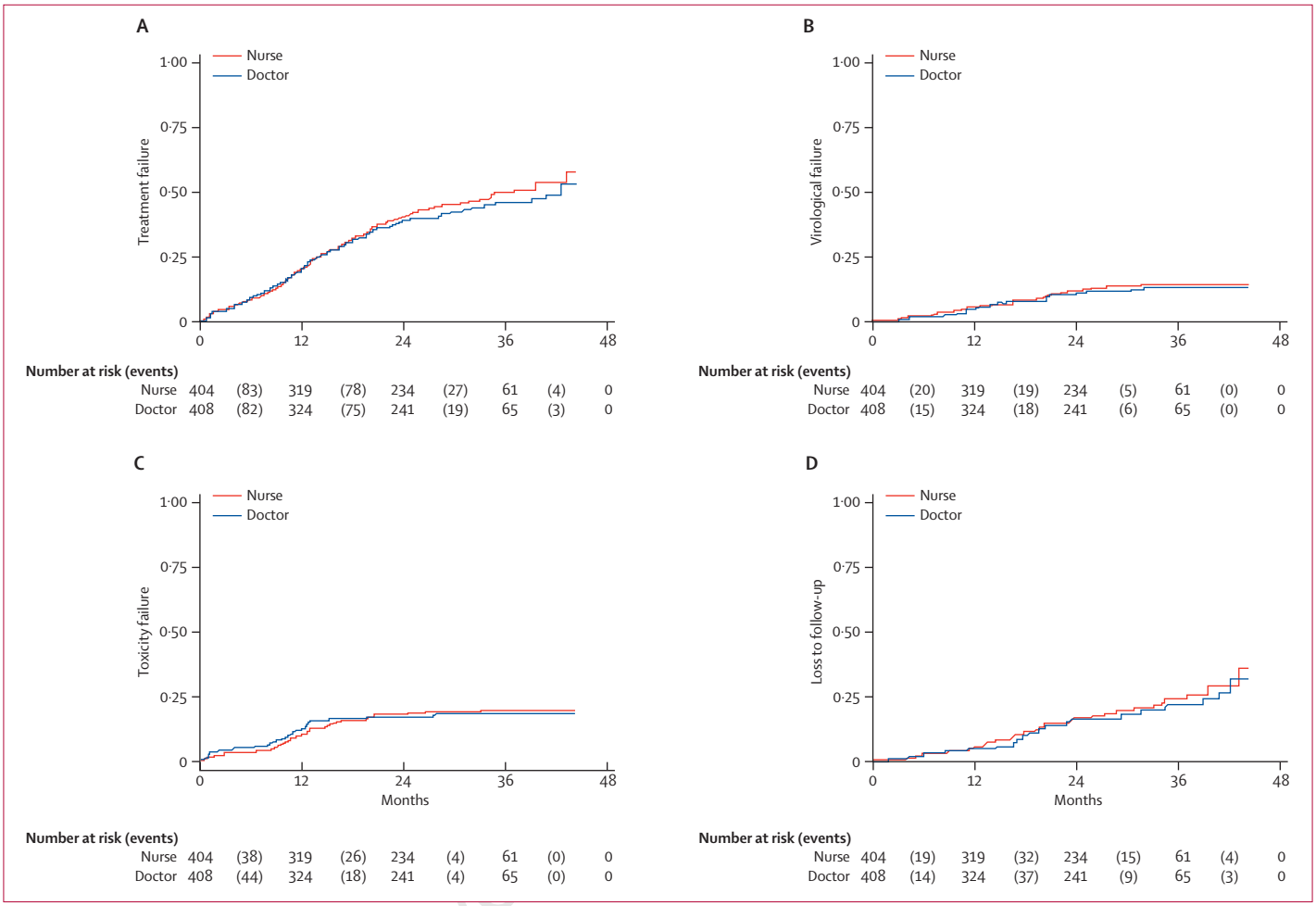

Figure 2: Kaplan-Meier curves of time to cumulative treatment failure (A) and of time to specific reasons for treatment failure (B–D) by study group
(A) Kaplan-Meier curve showing the composite endpoint of cumulative treatment failure. The primary health-care nurse group of the study was non-inferior to the doctor group (log-rank p=0·42). (B) Time to virological failure stratified by treatment group (log-rank p=0·53). (C) Time to toxicity failure stratified by treatment group (log-rank p=0·47). (D) Time to loss to follow-up stratified by treatment group (log-rank p=0·84).

4, 8, and 12, and every 12 weeks thereafter. Clinical records were maintained by the primary-care providers in each group. Study coordinators at each site extracted relevant study data into case report forms, which were relayed to a central database with Datafax (Clinical DataFax Systems, Hamilton, ON, Canada).

Statistical analysis

The sample size was calculated based on an 18-month accrual and 96 weeks' follow-up with 80% power and α of 0·05. Non-inferiority of the nurse group compared with the doctor group for cumulative treatment failure was prespecified as an upper 95% confidence limit for the hazard ratio (HR) that was less than 1·40. An initial sample size of 850 participants accounted for potential clustering of multiple enrolled participants within households. Because we did not record significant household clustering, enrolment was able to be discontinued after 812 patients with no compromise of pre-established study power.

Baseline differences in randomisation groups were described with simple proportions for categorical variables and means and SDs for continuous variables. The primary analysis was an intention-to-treat analysis of any treatment failure with use of Cox proportional hazards regression. Differences in specific reasons for treatment failure (eg, lost to follow-up, toxicity, death, etc) were compared by treatment group with HRs and 95% CIs. Differences in time to failure used Kaplan-Meier analyses. Group comparisons with the log-rank statistic were regarded as significant if p values were less than 0·05.

This study is registered with ClinicalTrials.gov, number NCT00255840.

Role of the funding source

Staff of the major sponsor of the study, the Division of AIDS (DAIDS) of the National Institutes of Allergy and Infectious Diseases, at the US National Institutes of Health, contributed to study design, data interpretation,

and review of the final report. The corresponding author had full access to all the data generated by the study and shared final responsibility for publication of the manuscript with IS.

Results

Figure 1 shows the trial profile. 917 participants were screened for study enrolment, of whom 828 met eligibility criteria and 812 consented and were randomly assigned. Of the 89 patients excluded from the study, 32 did not meet the ART initiation criteria, 22 had acute medical conditions, 18 were considered unsuitable by investigators or did not return, eight had laboratory results out of eligible range, and nine were unable to take oral drugs or were receiving excluded drugs. 804 (99%) study participants were black African and 573 (70%) were women. 408 individuals were randomly assigned to the nurse group and 404 to the doctor group. The median follow-up was 120 weeks (IQR 60–144), with no difference between the nurse and doctor groups (median 119 weeks [IQR 61–143] *vs* 120 weeks [58–144]). The follow-up period was 815·7 patient-years for the nurse group and 830·9 patient-years for the doctor group.

Table 1 shows baseline characteristics together with previous antiretroviral exposure and initial regimens. The study cohort had a median age of 32 years and had advanced HIV disease, as manifested by 282 (35%) of 812 patients having a previous CDC AIDS-defining event, viral loads greater than 100 000 copies per mL, and low median CD4 cell count (table 1). Patients in the nurse group were slightly more likely to be women and have CDC stage A disease than were those in the doctor group, but differences were small and not significant (table 1). Despite the slight preponderance of women in the nurse group, previous exposure to antiretroviral prophylaxis as part of mother-to-child prophylaxis was evenly distributed between study groups (table 1).

Most patients started with non-nucleoside-based therapy together with a nucleoside backbone of stavudine and lamivudine (table 1), in accordance with the prevailing South African national treatment guidelines.

The primary endpoint of cumulative treatment failure was reached by 371 (46%) patients after 1647 patient-years of follow-up (192 [48%] in the nurse group and 179 [44%] in the doctor group). With proportional hazards regression we recorded a 9% increased risk of failure in the nurse group (HR 1·09, 95% CI 0·89–1·33). The HR and 95% CI lie below the predefined study criterion for inferiority of 1·40. The time to composite failure estimated by Kaplan-Meier analysis was similar for each group (figure 2).

Figure 3 shows the HRs for individual treatment failure parameters of the composite endpoint. Deaths contributed 6% of the total events, viral failure 22%, toxicity failure 36%, and failure from protocol-defined loss to follow-up failure 36% (figure 3). The subcategories of the composite endpoint HRs were all closely distributed to 1·00 (95% CI 0·92–1·15). We recorded no significant differences between study groups for Kaplan-Meier estimates of time to death, viral failure, toxicity failure, and loss to follow-up (figure 2).

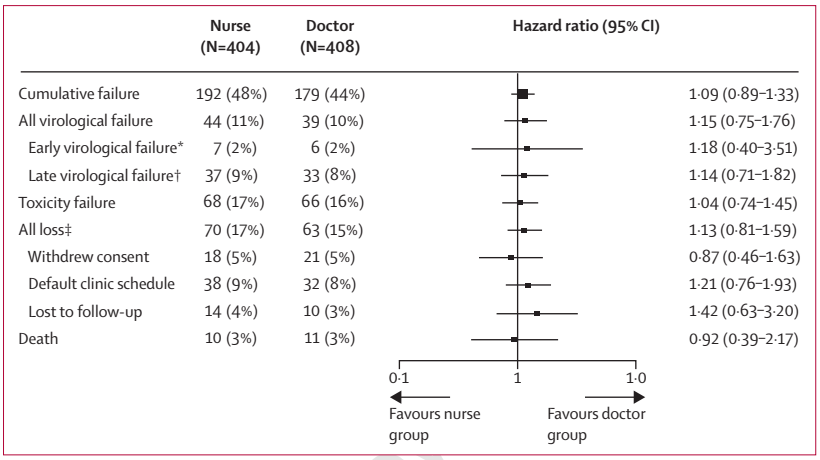

***Figure 3*: Cumulative treatment failure (primary endpoint) and accompanying reasons by study group**
The two groups of the study were compared with a composite endpoint or cumulative treatment failure. The composite consisted of each of the reasons listed below. *Early virological failure was defined as a decline of less than 1·5 $\log_{10}$ in viral load from baseline to 12 weeks of treatment. †Late virological failure was defined as two consecutive viral loads 4 weeks apart of more than 1000 copies per mL. ‡All loss was defined as: (1) withdrawal of consent, when the patient withdrew from participating in the study (in most cases a transfer away from the site to another location and clinic); (2) defaulting clinic schedule (a protocol-defined measure of adherence to the clinic schedule), for which any participant who missed three consecutive visits was considered to be defaulting; and (3) loss to follow-up, when a participant did not return to the clinic for three consecutive clinic visits and could not be traced .

21 deaths, ten in the nurse group and 11 in the doctor group, were included in the analysis. One further death in the doctor group was not included in the analysis because the participant had met a protocol-defined endpoint of toxicity failure before the death occurred. All deaths were reviewed by a masked endpoint review committee to assign the cause of death as study related, treatment related, disease related, or non-study related. Two deaths in the doctor group were assessed as due to lactic acidosis, and two deaths in the nurse group were considered to be non-study and non-HIV related. None of the participants met the protocol-defined criteria for disease progression.

Immune response was not a component of the primary endpoint; however, median increases in CD4 cell count from baseline were 155 cells (IQR 119–193) in the nurse group and 158 cells (125–169) in the doctor group at 1 year, and 239 cells (217–290) and 220 cells (174–274), respectively, at 2 years. Cumulative treatment failure was not affected significantly by either baseline CD4 cell count less than 200 cells per µL nor viral load more than 100 000 copies per mL (data not shown).

Table 2 shows grades 3 and 4 toxic effects that occurred during the study. The most frequent laboratory abnormalities were anaemia and neutropenia, raised lactate, and abnormal hepatic enzymes. The high frequency of hyperlactataemia resulted in a data safety monitoring board recommendation in 2007, for additional training and management of raised lactate.

	Nurse group		Doctor group		IRR (95% CI)
	Event in 815·74 person-years	Rate per 100 person-years (95% CI)	Event in 830·88 person-years	Rate per 100 person-years (95% CI)	
Laboratory events					
Haematology	62	7·6 (5·9–9·6)	106	12·7 (10·6–15·2)	0·60 (0·44–0·82)
Biochemistry	1	0·1 (0·0–0·6)	2	0·2 (0·0–0·8)	0·51 (0·05–5·62)
Liver	44	5·4 (4·0–7·1)	72	8·7 (6·9–10·7)	0·62 (0·43–0·91)
Hyperlactataemia	80	9·8 (7·9–12·0)	87	10·5 (8·5–12·7)	0·94 (0·69–1·27)
Pancreatic	4	0·5 (0·2–1·2)	4	0·5 (0·1–1·2)	1·02 (0·25–4·07)
Renal	3	0·4 (0·0–0·1)	1	0·1 (0·0–0·6)	3·06 (0·32–29·4)
Drug-related rash	4	0·5 (0·2–1·2)	5	0·6 (0·2–1·3)	0·81 (0·22–3·03)
Clinical HIV events					
Tuberculosis	28	3·4 (2·3–4·9)	31	3·7 (2·6–5·2)	0·92 (0·55–1·53)
Cervical dysplasia	1	0·1 (0·0–0·6)	4	0·5 (0·1–1·2)	0·25 (0·03–2·28)
Neurological	10	1·2 (0·6–2·2)	32	3·9 (2·7–5·3)	0·32 (0·16–0·65)
Intestinal	8	1·0 (0·5–1·9)	7	0·8 (0·4–1·7)	1·16 (0·42–3·21)
Skin	1	0·1 (0·0–0·6)	2	0·2 (0·0–0·8)	0·51 (0·05–5·62)
Lipodystrophy, lipoatrophy	45	5·5 (4·1–7·2)	51	6·1 (4·7–7·9)	0·90 (0·60–1·34)
Miscellaneous	23	2·8 (1·9–4·1)	23	2·8 (1·8–4·1)	1·02 (0·57–1·82)
Clinical non-HIV events					
CNS	5	0·6 (0·2–1·4)	13	1·6 (0·9–2·6)	0·39 (0·14–1·10)
Obstetric/gynaecology	10	1·2 (0·6–2·2)	7	0·8 (0·4–1·7)	1·46 (0·55–3·82)
Miscellaneous	34	4·2 (2·9–5·7)	37	4·5 (3·2–6·0)	0·94 (0·59–1·49)

The nurse group had 815·74 total person-years and the doctor group 830·88 person-years. Active reporting of adverse events was undertaken by the primary-care-giving nurse or doctor, and the study team undertook a retrospective review of all laboratory adverse events of grade 3 or greater. IRR=incidence rate ratio.

Table 2: **Rate of laboratory and clinical dose-limiting toxic effects, or grade 3 and 4 toxic effects as defined by the AIDS Clinical Trial Group,[28] by study group**

Grades 3 and 4 and dose-limiting toxic effects were more commonly reported in the doctor group than in the nurse group (incidence rate 1·31, 95% CI 1·14–1·49). However, a retrospective review by the CIPRA clinical safety team of all laboratory investigations undertaken throughout the study was consistent with the equal distribution of laboratory-defined adverse events between groups (data not shown). For clinical HIV-related (incidence rate ratio 0·32, 95% CI 0·16–0·65) and non-HIV-related neurological events (0·39, 0·14–1·10) doctors were more likely than were nurses to make a grade 3 or 4 neurological diagnosis.

Discussion

This study reports the findings of a prospective, randomised, controlled study comparing nurse-managed versus doctor-managed ART. A composite endpoint indicative of multiple aspects of ART delivery showed that nurse monitored therapy was not inferior to doctor monitored therapy. These findings lend support to observational data from other treatment programmes reporting successful use of task shifting in HIV care in both resource-limited (South Africa, Rwanda, and Lesotho)[28–31] and resource-rich (UK) countries,[32,33] and for other disease management.[34]

Expansion of ART services is urgently needed in resource-poor countries to achieve universal access targets by 2010,[35] and further expansion will be needed with the start of universal testing and treating strategies.[36] We noted no difference in mortality, viral failure, or immune recovery between the study groups. This study therefore lends supports to the strategy of task shifting, and suggests that HIV management by nurses can be safe and effective, probably even for those starting therapy with advanced HIV infection, although further studies with longer follow-up might be needed in this subgroup.

Although both study strategies successfully managed drug-related toxic effects, findings from this study do draw attention to a high frequency of lipomorphological changes and lactate increases associated with use of regimens including stavudine. WHO and South African guidelines have moved away from reliance on stavudine;[37,38] however, this drug remains widely used in resource-poor HIV therapy programmes.[12] In our study the overall frequency of drug-related toxic effects seemed to be lower than earlier reports of stavudine-based toxicities, which resulted in drug substitutions in excess of 20% after 3 years.[39] The dose reduction of stavudine to 30 mg after the first year of the study, which was in line with WHO recommendations,[27] might have reduced drug-limiting toxic effects. However, two of the study deaths were attributable to hyperlactataemia—a recognised complication of stavudine use.

Randomised controlled studies are frequently regarded as the gold standard on which treatment policies should be based. However, there might be some caveats in

application of trial findings to non-study settings and other populations. The study did not necessarily replicate the typical conditions under which therapy is delivered in resource-poor settings. For example, in addition to structured training in the use of ART, all clinical staff in the study received protocol-specific training in the conduct of ethical research including Good Clinical Practice, received didactic clinical management, and had access to ongoing telephonic clinical support if needed. However, widespread task shifting will need increased training, a redefinition of scope of practice for nurses, and a clinical support structure. The study results also cannot be generalised to settings where multiple first-line ART options might be used to individualise patient treatment, which in turn might reduce dose-limiting treatment toxic effects.

A strength of our study was that it was undertaken in busy primary-care clinics located in South African communities with a high burden of HIV, where large-scale task shifting will be needed. The cadre of nurses used in our study, primary-care nurses, are the staff whose role as clinician is being increasingly used in HIV and other specialties, such as tuberculosis in the South African health-care system. To restrict so-called contamination between the groups of the study, scheduled visits were booked for different days of the week once participants were randomly assigned. A weakness of the study is the limited time during which participants were on-study. The divergence of the groups might have continued over time, and the chance of noting divergence might have been limited by only 2 years of follow-up.

The study showed a high overall composite endpoint rate in both nurse and doctor groups. A stringent definition of treatment strategy failure included the traditional virological failure (10–11%), dose-limiting toxic effects (16–17% with stavudine regimens), death (3%), and all clinic losses (15–17%) that translate to failure of the treatment strategy to maintain patients on ART. These rates are similar to other studies despite our use of a more stringent definition of study loss, and use of a stavudine-based ART regimen with stringent criteria for hyperlactataemia and clinical toxic effects.[40] We noted a high rate of loss to follow-up in this study, but again this rate was similar to other studies in resource-constrained settings.[40] The future of large-scale antiretroviral programmes make it important to understand how this loss evolves over time. We noted some small differences in diagnosis of some grade 3 or 4 laboratory adverse events and some clinical diagnoses, which could have affected wider roll-out of nurse-based ART care. These differences could be addressed by more focused training on monitoring of laboratory data, and by implementation of simple algorithms of when further neurological work-up by a doctor is necessary.

The study design did not address nurse-initiated ART because the prescription of licensed drugs in South Africa is restricted to doctors. Implementation of nurse-initiated therapy would therefore need additional changes to the existing legislation. However, the new national HIV strategic plan does envisage initiation of therapy by doctors together with wide-scale task shifting to nurses for ongoing patient management.[20]

In conclusion, primary health-care nurses were non-inferior to doctors in monitoring of first-line ART in a public health ART programme in South Africa. The results of this study lend support to the expanded access to treatment with use of models of task shifting in primary health care.

Contributors

Each of the authors participated in the study design and protocol development. IS, RW, CO, PI, FC, JZ, and MR either undertook or supervised patient recruitment. RP, HT, CI, WS, and CH did laboratory and data management, and RG and MF did statistical design and analysis. All authors interpreted the data and reviewed the report, with the overall scientific oversight undertaken by IS, RW, and JMcI.

The CIPRA-SA Study Team

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Conflicts of interest

We declare that we have no conflicts of interest.

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Treatment interruption in a primary care antiretroviral therapy programme in South Africa: cohort analysis of trends and risk factors

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Abstract

Objective—To investigate antiretroviral treatment (ART) interruption in a long-term treatment cohort in South Africa.

Methods—All adults accessing ART between 2004 and 2009 were included in this analysis. Defaulting was defined as having stopped all ART drugs for more than 30 days. Treatment interrupters were patients who defaulted and returned to care during the study, whereas loss to follow-up was defined as defaulting and not returning to care. Kaplan-Meier estimates and Possion regression models were used to analyze rates and determinants of defaulting therapy and of treatment resumption.

Results—Overall rate of defaulting treatment was 12.8/100 person years (95% CI 11.4-14.4). Risk factors for defaulting were male gender, high baseline CD4 count, recency of ART initiation and time on ART. The probability of resuming therapy within 3 years of defaulting therapy was

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Role of the authors

KK designed the study, collected the data, and wrote the paper with input from NF, JL, JZ, CO, SDL and RW. KK designed and did the statistical analyses with input from JL and NF. JZ and CO oversaw the field site and RW was responsible for research infrastructure. All authors contributed to and approved the final version of the paper.

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42% (event rate=21.4/100 person-years). Factors associated with restarting treatment were female gender, older age, and time since defaulting.

Conclusion—Defaulting treatment need not be an irreversible event. Interventions to increase retention in care should target men, less immunocompromised patients and patients during the first 6 months of treatment. Resumption of treatment is most likely within the first year of interrupting therapy.

Keywords

HIV; antiretroviral; unstructured treatment interruption; loss to follow-up; Africa

Introduction

Access to antiretroviral therapy (ART) has improved substantially in resource-limited settings in Africa, Asia and South America where 90% of people with HIV/AIDS reside. According to World Health Organization (WHO) estimates, more than four million people with HIV/AIDS in low and middle income countries had initiated treatment by the end of 2008.[1] Despite this success, ensuring that patients remain in care over time remains one of the major challenges in resource-limited settings. Much attention has been paid to patient adherence[2-5], loss to follow-up, and mortality in ART programs in resource-limited settings.[6-9] A systematic review of 33 patient cohorts from 13 African countries reported that only between 46 and 85% of patients remained in care at two years.[8]

The realization that a substantial proportion of patients reported as lost to follow-up may have died has led to concern that there may be significant biases in program outcome reports of survival.[10] Another potential source of bias is the fact that a proportion of patients may only transiently default, returning to care at a later stage. Such unstructured treatment interruption has been reported to occur in around 20% of patients in industrialized settings.[11-14] The proportion of patients who transiently interrupt treatment in resource-limited settings is largely unreported.

Treatment interruptions, planned or otherwise, have been found to increase the risk of opportunistic infection and death[15-17], with viral load increase and associated CD4 decline most pronounced in the first two months.[16, 18-20] Interruptions raise similar concerns with respect to drug resistance and increased mortality as sub-optimal adherence.[11, 15, 21-23] However, few studies have addressed the issue of unstructured treatment interruptions in resource-limited settings. The aim of this study was to investigate the frequency and risk factors of defaulting treatment and identify factors associated with subsequent return to care in a long-term treatment cohort in South Africa.

Methods

Study site and data collection

The study was based in a peri-urban township in the greater area of Cape Town, with a population of approximately 15,000 people and an estimated adult HIV prevalence of 23% in 2005.[24] The community is served by a single public-sector primary care clinic which provides ART free of charge.

ART provision began in 2004. From 2005 to 2009 ART services were partly provided according to the Antiretroviral Treatment Protocol of the Western Cape and partly through a study funded by the National Institutes of Health (NIH). Patients enrolled in the NIH-funded study could access ART with a CD4 count below 350 cells/μl or WHO stage 3 disease as

compared to 200 cells/μl or WHO stage 4 disease in the provincial program. The NIH-funded study completed enrollment in 2007 after which all patients were treated in the provincial ART program.

Initial evaluation for ART eligibility included medical history, physical examination and CD4 cell count. A follow-up appointment was scheduled one to two weeks later when the laboratory results were reviewed and ART eligibility was determined. Patients eligible for ART underwent three adherence counseling sessions before starting treatment.

The initial follow-up schedule for those starting ART included one visit two weeks after ART initiation, followed by monthly visits until month three. Patients who were stable on ART and did not experience any adherence problems were thereafter seen every three months. Three attempts were made to contact patients who had missed appointments.

All patients aged ≥15 years accessing ART in the primary health care clinic between 01 March 2004 and 31 December 2009 were included in the analysis.

Sociodemographic and clinical data at baseline and laboratory data were collected prospectively using a standardized data form. All laboratory tests were performed by the National Health Laboratory Services in Cape Town.

Definitions

Patients defaulting treatment were defined as those who had not presented at the pharmacy for ART refills for more than 30 days. This category included patients who subsequently returned to care and restarted ART (treatment interrupters) and patients who had not returned to care at the time of censoring (loss to follow-up) (figure 1).

Treatment interruption was defined as a patient-initiated episode of more than 30 days of stopping ART (same definition as defaulting) but who subsequently resumed treatment (figure 1).

Patients lost to follow-up were those who stopped ART for more than 30 days and had not returned to care at the time of censoring (figure 1).

Study design

In-programme data on death, transfers outs and loss to follow-up were collected prospectively. Death on ART was defined as any death within three months of drug refill. If the exact date of death was not recorded it was estimated to be the 15th of the month following the last clinic appointment.

Patients who had stopped ART for more than 30 days and resumed therapy were identified using the pharmacy dispensing data. The electronic pharmacy dispensing system records each time medication is dispensed to a patient. Treatment interruption was verified through folder reviews.

The first endpoint was the time from ART initiation to the first time at which all drugs were stopped for a period of at least 30 days (default). Follow-up of patients on continuous therapy was censored at the date of death, date of transfer, or study end (31 December 2009).

The second endpoint was treatment resumption, defined as the time from defaulting treatment for the first time to the time of restarting ART. Follow-up of patients for whom therapy was not resumed was censored at the date of death, date of transfer, date of

migration, or study end. For a proportion of these patients (48%) vital status, date of death, date of transfer and date of migration was determined by home visits.

Statistical analysis

All analyses were carried out using Stata version 10.0 (Stata Corp. LP, College Station, TX, United States of America). Frequency tables were produced for all categorical baseline characteristics. For continuous baseline characteristics the median and interquartile ranges were reported. Standard survival analysis methods, including Kaplan-Meier estimates and Poisson regression models were used to analyze the rate and determinants of defaulting therapy and of treatment resumption after defaulting treatment for the first time. The proportional hazards assumption for potential interaction between each variable and time was tested using the likelihood ratio test. A univariate Poisson regression model was used to determine risk for time-to-event outcomes for each exposure variable. Multivariate models were built through backwards elimination. Sensitivity analyses were conducted excluding individuals with unascertained vital status. All reported p values are exact and 2-tailed, and for each analysis $p<0.05$ was considered significant.

Ethical approval

The study was approved by the University of Cape Town Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. Written informed consent was obtained from all patients at enrolment.

Results

Patient characteristics

A total of 1154 patients were included in the analysis (table 1) and the median time of follow-up was 1.45 years (IQR: 0.48-3.24). The majority of patients were young women (65.2%) and residents in the township (95.5%). Prior to treatment initiation the majority of patients were in WHO clinical stage 3 (51.1%) and 4 (25.1%) and median CD4 count was 122 cells/μl (IQR: 54-190). The number of patients initiating ART per year doubled from 137 in 2004 to 279 in 2006 and declined thereafter.

A total of 291 patients defaulted treatment at least once (figure 1). Among these, 96 resumed therapy (treatment interruption) while 195 did not resume therapy during follow-up (lost to follow-up). Of the 96 individuals resuming therapy, 75 individuals had one episode of treatment interruption, 19 had two and 2 had three. The median time patients failed to receive ART was 228 days (IQR: 126-409) during the first episode of treatment default and 194 days (IQR 121-278) during the second episode. Thirty five patients who had stopped treatment underwent re-screening that included clinical assessment, laboratory tests and adherence counseling and yet did not resume therapy during the period of the study.

Subsequent analyses investigated first episode of treatment interruption by analyzing the time to stopping treatment for the first time and resuming therapy thereafter (non-shaded cells in figure 1).

Factors associated with the probability of defaulting treatment

The overall rate of treatment default for the first time was 12.8/100 person years (PYs) (95% CI 11.4-14.4). The Kaplan-Meier estimate of the probability of defaulting treatment for at least 30 days was 14.9% (95% CI 12.7-17.4) by 1 year, 25.6% (95% CI 22.7-28.8) by 2 years and 41.0% (95% CI 37.0-45.3) by 5 years from ART initiation (figure 2).

Factors associated with increased risk of defaulting therapy in univariate analysis were male gender, higher baseline CD4 count, recency of ART initiation and shorter duration on ART (table 2). Defaulting rate was highest in the first six months of ART (18.2/100 PYs, 95% CI 14.7-22.5) but decreased thereafter and had more than halved after two years (8.8/100 PYs, 95% CI 7.0-11.0).

Gender, baseline CD4 count, time on ART, and date of initiation remained significantly associated with defaulting in the multivariate model. Men were 1.51 (95% CI 1.18-1.93) times more likely to default treatment compared to women, as were those patients with a higher baseline CD4 count. The adjusted hazard of defaulting treatment increased by 1.30 (95% CI 1.17-1.44) for each calendar year. Patients on treatment for more than 2 years had a lower hazard of 0.69 (95% CI 0.48-0.98) of defaulting compared to patients during the first 6 months of treatment. Similar results were found in a sensitivity analysis that excluded individuals whose vital status could not be ascertained.

Factors associated with the probability of resuming therapy

A total of 291 patients defaulted treatment at least once. The overall rate of treatment resumption after defaulting treatment for the first time was 21.4/100 PYs (95% CI 17.5-26.2) (figure 3). The Kaplan-Meier cumulative estimate of the probability of treatment resumption was 26.7% (95% CI 21.7-32.7) in the first year, 37.1% (95% CI 31.1-43.9) in the second year and 42.1% (95% CI 35.2-49.7%) in the third year after stopping treatment.

In univariate analysis a greater likelihood of resuming ART was associated with older age and shorter time since defaulting (table 3); gender, residency, calendar year of defaulting and CD4 count nearest to the time of defaulting was not associated with resuming treatment.

In multivariate analysis men were less likely to resume treatment compared to women (IRR 0.67, 95% CI 0.43-1.04, p=0.07); whereas patients >30 years old were more likely to restart treatment (IRR 1.80, 95% CI 1.13-2.89). The likelihood of resuming treatment decreased significantly beyond one year of defaulting treatment (IRR 0.40, 95% CI 0.25-0.63).

Of the 96 patients resuming therapy, 86 had a CD4 count measurement while receiving therapy and before the treatment interruption; the majority of these (80) responded to ART with an increase in CD4. Patients who resumed therapy were found to have a median CD4 count (150.5 cells/μl, IQR: 73-266) comparable to their baseline CD4 count prior to initiating therapy (138.5 cells/μl, IQR: 73-188). The median time between the measurement of CD4 count and resuming therapy was 13 days (IQR 0-28 days).

Excluding individuals with unascertained vital status revealed similar results with regards to parameter estimates, but the association with male gender became non-significant (HR 0.81, 95% CI 0.52-1.26, p=0.35).

Discussion

To our knowledge, this is the first study from sub-Saharan Africa to report on unstructured treatment interruptions in a routine programme setting. Our analysis shows that treatment interruption is a common phenomenon. The probability of ART defaulters to resume therapy within 3 years was 42%. Most ART cohorts report on loss to follow-up defined as not attending the clinic for more than 3 months,[8] and assume that loss to follow-up is an irreversible event. Our study shows that patients who fulfil the widely used definition of loss to follow-up at one time point might resume therapy later. In this cohort, the median duration of the first treatment interruption was 7.5 months.

The median CD4 count of those resuming therapy was similar to their initial CD4 count prior to starting treatment, which underscores the potentially negative impact of interruption leading to a reversal in immunological recovery made while on treatment. Data from industrialised settings suggest that treatment interruption has detrimental effects on CD4 count, viral load suppression, and clinical progression.[11, 12, 19] Programmes that report patient attrition and the number of patients in care will not account for the potential that up to 14% of patients in care have interrupted treatment at least once.

We were able to determine risk factors for defaulting ART and factors associated with resuming therapy. Male gender, high baseline CD4 count, recency of ART initiation and the first 6 months of treatment were associated with a higher risk of defaulting. Treatment resumption was more likely in women, patients more than 30 years old and within the first year of stopping therapy.

Our finding that men were at higher risk of defaulting treatment and less likely to resume treatment is consistent with studies showing that HIV-infected men are less likely to access treatment[25, 26], have an increased risk for loss to follow-up in the pre-treatment period[27], present with more advanced stages of HIV disease[28] and have a higher mortality risk on ART.[2, 9, 29-33] Strategies to diagnose HIV in men earlier and to link and to retain them in care might include: i) extending clinic hours into evenings and weekends, ii) training male health care staff and counsellors, iii) offering additional adherence sessions to men and iv) initiating male support groups.

Individuals initiating treatment in more recent years were more likely to default, suggesting that programmatic factors might influence retention in care. A study including data from 15 treatment cohorts from Africa, Asia and South America showed that early patient losses were increasingly common when programs were scaled up.[6] Increasing cohort size in an environment of scarce human resources for health has been suggested to influence both the scale-up capacity and the long-term retention in ART programs.[34] In the study clinic resources and staffing were further reduced when enrolment for the NIH-funded study finished in 2007. In contrast, year of defaulting was not associated with resumption of treatment, suggesting that patient tracing was less influenced by cohort size (although this would vary according to tracing procedures).

Treatment defaulting was more likely in patients with less advanced immunodeficiency at baseline. This may be explained by the fact that individuals who default treatment and stay alive do so because they feel better on treatment, a phenomenon that has been reported by other studies.[35] This finding is particularly important in view of the 2009 WHO guidelines recommending ART initiation at CD4 counts below 350 cells/ μl[36] and when considering initiation of ART regardless of CD4 count as proposed in the 'test and treat' strategy.[37] Initiating ART at the time of HIV diagnosis will result in increased numbers of relatively immunocompetent individuals on ART who may have a higher risk of defaulting treatment. Specific interventions aimed at these individuals need to be developed to ensure optimal retention in care.

This study has several limitations. First, ascertainment of vital status for treatment defaulters was incomplete, which may have led to a misclassification of deaths as defaulters. However, sensitivity analysis excluding individuals with unascertained vital status did not influence our overall findings. Second, resumption of therapy was not ascertained in patients who moved to other communities, possibly resulting in underestimation of treatment resumption. Third, the clinical and immunological consequences of treatment interruption were not analysed due to lack of laboratory data, in particular the lack of capacity to perform routine viral load, and the small number of individuals resuming therapy. However it has been

shown in industrialised settings that treatment interruption impacts negatively on CD4 count, viral load suppression and clinical progression.[11, 12, 19]

We consider that the main finding of this study that a considerable proportion of treatment defaulters return to care is likely to be generalisable to similar settings. Nevertheless, risk factors for defaulting and resuming therapy might differ with regards to eligibility criteria and resources available for patient tracing.

A strength of this study is that the relatively large sample size and follow up time. This allows for an assessment of risk factors for defaulting and treatment interruption that in turn allows for several proposals to be made to limit defaulting and treatment interruption in similar programme settings. In particular, interventions to keep patients in care should be targeted at men, patients with higher CD4 counts and during the first 6 months of ART. Moreover, the finding that the probability of resuming therapy was highest in the first year following treatment defaulting suggests that efforts to bring patients back into care might be most successful early into defaulting treatment.

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Figure 1. Flow chart of patients in care, first time defaulters and treatment interrupters

[1] Defaulting treatment was defined as having stopped all ART drugs for more than 30 days. This category included patients who subsequently returned to care and restarted ART (treatment interrupters) and patients who had not returned to care at the time of censoring (loss to follow-up).

[2] Loss to follow-up was defined as stopping ART for more than 30 days and not returning to care at the time of censoring.

[3] Treatment interruption was defined as a patient-initiated episode of more than 30 days of stopping ART and subsequently resuming treatment.

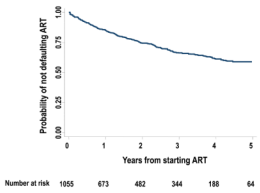

Figure 2.
Kaplan Meier plot showing the probability of not defaulting antiretroviral therapy (ART) from the time of initiating ART up to the end of the 5th year of treatment

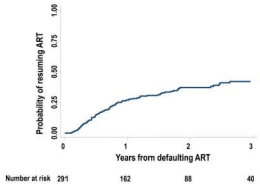

Figure 3.
Kaplan Meier plot showing the probability of resuming antiretroviral therapy (ART) from the time of defaulting therapy up to 3 years after defaulting treatment

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Table 1

Baseline characteristics of patients (n=1154) who enrolled in the ART programme between 2004 and 2009

Variable		N (%)	Median (IQR)
Gender	Women	752 (65.2)	
	Men	402 (34.8)	
Age (years)			31.9 (27.3; 37.5)
Residents in the study township	Yes	1102 (95.5)	
	No	40 (3.5)	
	Unknown	12 (1.0)	
Transferred in from another ART service	No	1046 (90.6)	
	Yes	108 (9.4)	
Year of initiating ART	2004	137 (11.9)	
	2005	242 (21.0)	
	2006	279 (24.2)	
	2007	153 (13.3)	
	2008	155 (13.4)	
	2009	188 (16.3)	
WHO clinical stage[1]	1	106 (9.3)	
	2	166 (14.5)	
	3	585 (51.1)	
	4	287 (25.1)	
Baseline CD4 (cell/μl)[2]			122 (54; 190)

[1] 10 missing values

[2] 114 missing values

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Table 2

Risk factors for defaulting treatment

Variable		Number defaulting treatment	Person years at risk	Rate of default of treatment per 100PYs (95% CI)	Unadjusted HR of default of treatment (95% CI)	P valiue	Adjusted HR of default of treatment (95% CI)	P value
Gender	Women	172	1544	11.1 (9.6-12.9)	1		1	
	Men	115	692	16.6 (13.9-20.0)	1.49 (1.17-1.89)	<0.01	1.51 (1.18-1.93)	<0.01
Age (years)	≤30	196	1473	13.3 (11.6-15.3)	1			
	>30	91	762	11.9 (9.7-14.7)	0.90 (0.70-1.15)	0.40		
Residents in the study township	Yes	271	2168	12.5 (11.1-14.1)	1			
	No	10	65	15.4 (8.3-28.7)	1.23 (0.66-2.32)	0.52		
Transferred from another ART service	No	268	2110	12.7 (11.3-14.3)	1			
	Yes	19	127	15.0 (9.6-23.5)	1.18 (0.74-1.88)	0.48		
WHO stage	1 or 2	57	499	11.4 (8.8-14.8)	1			
	3 or 4	209	1608	13.0 (11.4-14.9)	1.14 (0.85-1.53)	0.37		
Baseline CD4 count (cells/μ)	≤100	89	823	10.8 (8.8-13.3)	1		1	
	101-200	103	716	14.4 (11.9-17.3)	1.33 (1.00-1.77)	0.05	1.32 (0.99-1.76)	0.06
	>200	73	530	13.8 (10.9-17.3)	1.27 (0.83-1.73)	0.13	1.39 (1.02-1.91)	0.04
Year of initiating ART[1]	2004	24	434	5.5 (3.7-8.2)				
	2005	76	703	10.8 (8.6-13.5)				
	2006	83	599	13.9 (11.2-17.2)	1.36 (1.24-1.48)	<0.01	1.30 (1.17-1.44)	<0.01
	2007	47	247	19.0 (14.3-25.3)				
	2008/2009	57	253	22.6 (17.4-29.3)				
Time on ART	<6months	84	462	18.2 (14.7-22.5)	1		1	
	6months-2year	130	939	13.8 (11.7-16.4)	0.76 (0.58-1.00)	0.05	0.86 (0.65-1.15)	0.31
	>2years	73	834	8.8 (7.0-11.0)	0.48 (0.35-0.66)	<0.01	0.69 (0.48-0.98)	0.04

[1] p value for test for departure from linear trend 0.35

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Table 3

Risk factors for resuming treatment after defaulting

Variable		Number resuming treatment	Person years at risk	Rate of restarting treatment per 100PYs (95% CI)	Unadjusted IRR of restarting treatment (95% CI)	P value	Adjusted IRR of restarting treatment (95% CI)	P value
Gender	Women	61	253	24.1 (18.7-31.0)	1		1	
	Men	32	182	17.6 (12.5-24.9)	0.73 (0.48-1.12)	0.15	0.67 (0.43-1.04)	0.07
Age (years)	≤30	26	174	15.0 (10.2-22.0)	1		1	
	>30	67	261	25.7 (20.2-32.6)	1.72 (1.09-2.70)	0.02	1.80 (1.13-2.86)	0.01
Residents	Yes	87	405	21.5 (17.4-26.5)	1			
	No	5	14	34.5 (14.4-82.9)	1.60 (0.65-3.96)	0.30		
CD4 count at time of defaulting (cells/μ)	≤200	36	155	23.4 (16.8-32.4)	1			
	> 200	57	280	20.3 (15.7-26.3)	0.87 (0.57-1.32)	0.52		
Year of defaulting treatment	2004/2005	9	60	15.0 (17.8-28.9)	1			
	2006	23	122	18.8 (12.5-28.3)	1.25 (0.58-2.70)	0.57		
	2007	29	127	22.7 (15.8-32.6)	1.51 (0.72-3.19)	0.28		
	2008	32	93	21.4 (13.8-33.2)	1.42 (0.65-3.13)	0.38		
	2009	12	31	38.5 (21.9-67.8)	2.57 (1.08-6.09)	0.03		
Time off ART	<1 year	68	222	31.6 (24.2-38.9)	1		1	
	>1year	25	213	11.7 (7.9-17.4)	0.38 (0.24-0.61)	<0.01	0.40 (0.25-0.63)	<0.01

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Linkage to HIV Care and Antiretroviral Therapy in Cape Town, South Africa

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Abstract

Background: Antiretroviral therapy (ART) has been scaled-up rapidly in Africa. Programme reports typically focus on loss to follow-up and mortality among patients receiving ART. However, little is known about linkage and retention in care of individuals prior to starting ART.

Methodology: Data on adult residents from a periurban community in Cape Town were collected at a primary care clinic and hospital. HIV testing registers, CD4 count results provided by the National Health Laboratory System and ART registers were linked. A random sample (n = 885) was drawn from adults testing HIV positive through antenatal care, sexual transmitted disease and voluntary testing and counseling services between January 2004 and March 2009. All adults (n = 103) testing HIV positive through TB services during the same time period were also included in the study. Linkage to HIV care was defined as attending for a CD4 count measurement within 6 months of HIV diagnosis. Linkage to ART care was defined as initiating ART within 6 months of HIV diagnosis in individuals with a CD4 count ≤200 cells/μl taken within 6 months of HIV diagnosis.

Findings: Only 62.6% of individuals attended for a CD4 count measurement within 6 months of testing HIV positive. Individuals testing through sexually transmitted infection services had the best (84.1%) and individuals testing on their own initiative (53.5%) the worst linkage to HIV care. One third of individuals with timely CD4 counts were eligible for ART and 66.7% of those were successfully linked to ART care. Linkage to ART care was highest among antenatal care clients. Among individuals not yet eligible for ART only 46.3% had a repeat CD4 count. Linkage to HIV care improved in patients tested in more recent calendar period.

Conclusion: Linkage to HIV and ART care was low in this poor peri-urban community despite free services available within close proximity. More efforts are needed to link VCT scale-up to subsequent care.

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Introduction

South Africa is home to one-sixth of the world's population living with HIV and has the largest antiretroviral therapy (ART) programme in the world [1,2]. ART roll out began nationally in late 2003 and by the middle of 2008, 568,000 adults and children were receiving ART. This translated into around 40% of eligible adults receiving ART in 2008 [3], although the latest guidelines recommend earlier initiation for certain patients, thus increasing the numbers eligible for ART and widening the treatment gap [4].

In an effort to increase access to prevention and care, South Africa launched an ambitious national campaign in April 2010 aiming to test 15 million people for HIV and to reach 1.5 million people with ART by June 2011. Increased HIV testing may impact on risk behavior in the short-term [5]. However, there is also a need to ensure that those who need treatment are linked to the appropriate services while those not eligible for treatment are monitored and started on ART when appropriate. A study from Durban, South Africa, reported that almost two-thirds of newly diagnosed patients accessing care in a semi-private hospital were lost to care between HIV diagnosis and getting a CD4 count, and another one in five patients were lost between CD4 testing and ART initiation [6,7]. Another study from South Africa found that only 45% of eligible patients started ART in a public sector ART project in Free State. Mortality and TB incidence in patients failing to initiate ART was more than 2 times higher compared to patients initiating ART [8].

The impact of ART on mortality, morbidity, TB incidence [9,10] and HIV transmission [11] at a population level depends on ART coverage. ART coverage defined as the number of patients

receiving ART at a point in time, divided by the number needing treatment is determined by timely HIV diagnosis and effective linkage to ART. This study investigates linkage to HIV and ART care using a random sample of individuals testing HIV positive either provided-initiated (through antenatal care (ANC), tuberculosis (TB), sexually transmitted infection (STI) services) or client-initiated (through voluntary counseling and testing (VCT) services) in a peri-urban township in the Western Cape Province in South Africa. Linkage to care was defined first as attending for a CD4 count measurement within 6 months of a positive HIV test and second as the proportion of eligible individuals starting ART within 6 months of their HIV diagnosis.

Methods

Setting

The study was based in a peri-urban township in the greater area of Cape Town, with a population of approximately 15,000 people and a measured adult HIV prevalence of 23% in 2005 [12]. The community is served by a single public-sector primary care clinic, which provides outpatient care including ART free of charge. A nearby hospital (5 km away) provides all secondary care for the population, including inpatient and antenatal services. The hospital also provides ART for some HIV-infected individuals from the community.

HIV testing, CD4 count measurements and ART services

Client-initiated HIV testing services have been available to all individuals accessing either the local clinic or the hospital since 2001. Clients who tested on their own initiative are referred to as having tested through VCT services. Provider-initiated testing was routinely provided to any patient accessing TB services whose HIV status was unknown. This was extended to all pregnant females accessing the hospital or clinic in 2002 and patients accessing STI services in 2007. All testing required signed consent. All CD4 count tests were free for patients and performed by the centrally located National Health Laboratory Services (NHLS) in Cape Town.

ART provision at the primary health care clinic and hospital began in 2004.

Linkage to HIV and ART care

Linkage to HIV care was defined as attending for a CD4 count measurement within 6 months of HIV diagnosis. We did not ascertain if individuals actually received their CD4 counts. Linkage to ART care was defined as initiating ART within 6 months of HIV diagnosis in individuals with a CD4 count $\leq$200 cells/μl taken within 6 months of HIV diagnosis. Having a repeat CD4 count was defined as having had a repeated CD4 count in individuals not yet eligible for ART (CD4 count >200 cells/μl) and tested before 2009.

Data collection

We collected data from 3 sources. First, the primary care clinic and hospital HIV testing registers provided all data on HIV infected, adult community residents ($\geq$18 years) diagnosed between January 2004 and March 2009. Data at the primary health care clinic were missing for the period from February 2008 to August 2008. For each test encounter recorded in the registers, we retrieved data on client identification variables (first name, surname, date of birth, and medical record number); place of residence; sex; test acceptance; test result and service. For HIV infected individuals who tested more than once, the earliest positive HIV test was considered. Second, data on CD4 counts performed at either the primary care clinic or the hospital in the period from 2004 to October 2009 were obtained from NHLS. The date of CD4 count was the date the client provided blood. Third, data from residents who initiated ART care at the primary health care clinic or hospital were obtained from electronic ART registers at the clinic and hospital.

These three databases were merged on first name, surname, medical record number and date of birth. In cases where identifiers did not match completely two researchers (PG and KK) independently confirmed that records in different databases were from the same individual. Concordance between the two researchers was 97%. Cases where the two researchers disagreed were discussed until consensus was reached. For all subsequent analysis data was stripped of all personal identifiers.

Ethics

Written informed consent was obtained from all individuals initiated on ART and screened for ART. Individuals testing for HIV are routinely entered into the HIV testing register. Informed consent was not obtained from HIV positive individuals not linking to care, as this was a retrospective study and individuals were not actively follow-up. Data collection and analysis was approved by the University of Cape Town Ethics Committee and Partners Human Subjects Institutional Review Board and the London School of Hygiene and Tropical Medicine.

Statistical analysis

A random sample (n = 885) of adults testing HIV positive through ANC, STI and VCT services between January 2004 and March 2009 was selected for this analysis. All adults testing positive through TB services were included in this analysis to ensure an adequate sample size in this group.

All analyses were carried out using Stata version 11 (Stata Corp. LP, College Station, TX, United States of America). Proportions were calculated stratified by service. Total proportions were calculated taking the different sampling proportions into account. Risk ratios investigating associations between age, sex, calendar period and timely linkage to HIV care, CD4 count $\leq$200 cells/μl and repeated CD4 counts were estimated for each service. Risk ratios were calculated using a log binominal model [13].

Results

HIV testing and HIV prevalence

A total of 8515 records of HIV tests were available for adult members of the community. The majority of individuals tested through VCT (n = 5345, 62.8%) services (Table 1). The overall HIV prevalence among those tested was 23.5% with the highest prevalence among patients tested through TB (37.9%) and VCT services (24.9%) (χ^2 test, $p<0.01$). The median age of individuals tested was 26 (interquartile range (IQR), 22–32) and 67.9% were women. HIV prevalence was 21.6% in men and 24.4% in women.

A total of 2002 clients tested HIV positive. Their median age was 28 years (IQR, 24–33) and the majority were women (70.3%). The proportion of women testing HIV positive was 100% in ANC, 66.2% in STI, 38.8% in TB and 66.4% in VCT clients. 1330 (66.4%) individuals tested HIV positive through VCT, 332 (16.6%) through ANC, 237 (11.8%) through STI and 103 (5.1%) through TB services.

Linkage to HIV and ART care

Linkage to HIV and ART care was assessed in a random sample of 47% of individuals testing HIV positive through ANC, STI and VCT services and 100% of individuals testing through TB services: 150 tested through ANC, 113 through STI, 662 through VCT and 103 through TB services. Only 62.6% (95%CI

Table 1. Number (%) of individuals who tested for HIV and who were found to be positive stratified by type of clinical service.

	ANC service	STI service	TB service	VCT service	Total
Tested N (%)	1525 (17.9)	1370 (16.1)	275 (3.2)	5345 (62.8)	8515 (100)
Positives N (%)	332 (16.6)	237 (11.8)	103 (5.1)	1330 (66.4)	2002 (100)
HIV Prevalence	21.8%	17.3%	37.5%	24.9%	23.5%

All HIV testing records available for the period from January 2004 until March 2009 from adult patients were included in this analysis.
ANC = antenatal care, STI = sexual transmitted infections, TB = tuberculosis, VCT = voluntary counseling and testing.
doi:10.1371/journal.pone.0013801.t001

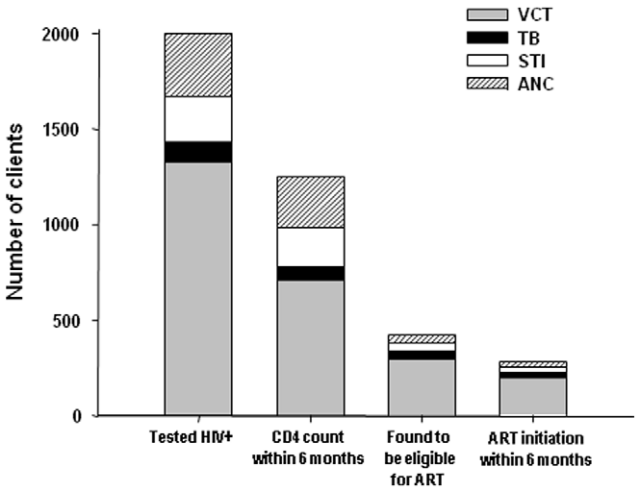

Figure 1. Number of clients testing HIV+, with timely CD4 counts, eligible for ART and initiating ART estimated using proportions from table 2. ART = antiretroviral therapy, ANC = antenatal care, STI = sexual transmitted infections, TB = tuberculosis, VCT = voluntary counseling and testing.
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59.6–65.5) of clients attended for a CD4 count measurement within 6 months of testing HIV positive (Table 2) and 26.3% (95%CI 23.5–29.0) did not have any recorded CD4 count test. The proportion of individuals attending for a CD4 count measurement within 6 months was highest among individuals tested through ANC (81.3%) and STI (84.1%) services and lowest among those who learnt of their status via VCT (53.5%) (Table 2).

Among individuals with a CD4 count measurement within 6 months, 34.1% (95%CI 30.4–37.7) were eligible for ART according the South African Department of Health criteria (CD4 count ≤200 cells/μl) at the time of the study (Table 2). Low CD4 counts were more prevalent among individuals tested through TB (54.9%) and VCT services (42.6%). In individuals attending for a CD4 count measurement within 6 months the median time between HIV test and CD4 count measurement was: 2 days (IQR 2–6) for ANC, 3 days (IQR 2–4) for STI, 3 days (IQR 2–5) for TB and 2 days (IQR 2–4) for VCT clients. Overall 4.3% of clients attended for a CD4 test at the same day as the HIV test. The majority of clients attended for CD4 count testing within 1 week (84.9%), 14.2% within 8 days and 3 months and only 0.9% within 3 and 6 months.

In individuals with a delayed first CD4 count measurements, the mean time between HIV diagnosis and first CD4 count was 490 days (IQR 345–769). Among patients with delayed first CD4 count measurements, 33.2% (95%CI 24.3–42.1) had a CD4 count ≤200 cells/μl and 26.2% (95%CI 17.8–34.8)−43.2) had a CD4 count of 201–350 cells/μl.

Only 66.7% (95% CI 60.2–73.1) of eligible individuals with a timely CD4 count accessed ART care within 6 months of HIV testing (Table 2). Linkage to ART care was highest among individuals tested through ANC services (72.2%). Among individuals not yet eligible for ART only 46.3% (95%CI 41.4–51.1) ever had a repeat CD4 count. Median time between the first and the second CD4 count was 236 days.

Figure 1 summarizes the number of people tested through different services and the numbers linking to HIV and ART care by service using the proportions estimated from the random sample.

Table 2. Percentage of individuals linking to HIV care (as defined by attending for a CD4 cell count measurement), distribution of CD4 count measurements, percentage of patients subsequently initiating ART and percentage of clients non-eligible for ART returning for a repeat CD4 count.

Variables		ANC (n = 150) % (N)	STI (n = 113) % (N)	TB (n = 103) % (N)	VCT (n = 622) % (N)	Total %
First CD4 count after HIV test	**≤6 months**	81.3 (122)	84.1 (95)	68.9 (71)	53.5 (333)	62.6 (59.6–65.5)
	>6 months	2.0 (3)	2.7 (3)	13.6 (14)	14.8 (92)	11.1 (9.2–13.1)
	None	16.7 (25)	13.3 (15)	17.5 (18)	31.7 (197)	26.3 (23.5–29.0)
First CD4 count within 6months of HIV test	**≤200 cells/μl**	14.8 (18)	22.1 (21)	54.9 (39)	42.3 (141)	34.1 (30.4–37.7)
	201–350 cells/μl	24.6 (30)	32.6 (31)	23.9 (17)	23.7 (79)	25.3 (21.8–28.8)
	>351 cells/μl	60.7 (74)	45.3 (43)	21.1 (15)	33.9 (113)	40.6 (36.8–44.4)
ART initiation within 6 months of HIV test in eligible individuals with timely first CD4 count	**Yes**	72.2 (13)	52.4 (11)	71.8 (28)	67.4 (95)	66.7 (60.2–73.1)
	No	27.8 (5)	47.6 (10)	28.2 (11)	32.6 (46)	33.3 (26.9–39.*)
Repeat CD4 count in individuals with a first CD4 count >200 cells/μl	**Yes**	48.5 (47)	57.6 (38)	34.5 (10)	42.3 (96)	46.3 (41.4–51.1)
	No	51.5 (50)	42.4 (28)	65.5 (19)	57.7 (131)	53.7 (48.9–58.6)

ANC = antenatal care, STI = sexual transmitted infections, TB = tuberculosis, VCT = voluntary counseling and testing.
doi:10.1371/journal.pone.0013801.t002

Table 3. Factors associated with linkage to HIV care (attending for a CD4 count measurement within 6 months of HIV diagnosis) stratified by service.

Variables	ANC	STI	TB	VCT
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Female	NA	1	1	1
Male	NA	0.93 (0.79–1.09)	1.01 (0.82–1.25)	1.10 (1.01–1.33)
Age<30 years	1	1	1	1
Age≥30 years	0.97 (0.90–1.04)	1.17 (1.01–1.35)	1.07 (0.84–1.35)	1.16 (0.96–1.26)
Tested in 2004–2006	1	NA	1	1
Tested in 2007–2009	**0.87 (0.74–0.99)**	NA	**1.67 (1.27–2.21)**	**1.60 (1.40–1.84)**

ANC = antenatal care, STI = sexual transmitted infections, TB = tuberculosis, VCT = voluntary counseling and testing.
NA = not applicaple.
doi:10.1371/journal.pone.0013801.t003

Predictors of low CD4 count, linkage to HIV, and repeated CD4 counts

Risk ratios investigating predictors for linkage to HIV care showed that linkage to care in TB (RR 1.67, 95%CI 1.27–2.21) and VCT (RR 1.60, 95%CI 1.40–1.84) clients was more likely in 2007–2009 compared to 2004–2006 (table 3). This was not the case for ANC (RR 0.97) clients who were slightly less likely to link to HIV care if tested more recently (table 3). Linkage to ART care could only be assessed in VCT clients due to the small sample size in the other groups. Neither age (RR 0.85, 95%CI 0.67–1.09) nor sex (RR 1.03, 95% CI 0.81–1.31) nor year of testing (RR 1.02, 95% CI 0.81–1.30) predicted linkage to HIV care in VCT clients.

The risk of having CD4 count measurement ≤200 cells/μl was higher in individuals aged more than 30 years regardless which service they tested through (table 4). Repeated CD4 counts were 1.3 times more likely in individuals more than 30 years of age, but this result only reached significance in the VCT clients (RR 1.25, 95% CI 1.00–1.55).

Discussion

This study evaluated the proportion of individuals linking to HIV care in a public sector service in Cape Town, South Africa. Only 63% of patients attended for a CD4 count measurement within 6 months of diagnosis. Although a substantial proportion of patients had CD4 counts ≤200 cells/μl (34%) and were therefore eligible for ART according to South African guidelines [14], only 67% of these started ART within 6 months. Among those who did have a timely CD4 count but were not yet eligible for ART, only 46% returned for a repeat CD4 count after a median time of 8 months. Individuals testing through ANC services had better linkage to HIV and ART care and higher CD4 counts at time of HIV diagnosis compared to individuals accessing the other services.

HIV is a chronic disease and comprehensive HIV care needs to be provided within a continuum of care [15]. ART is just one of the components of HIV care and care of individuals not yet requiring ART is equally important [16]. The continuum of HIV care starts when an individual is diagnosed with HIV. ART eligibility should be assessed when individuals are newly diagnosed and in regular (6 monthly) intervals thereafter. Individuals not yet eligible for ART should receive comprehensive HIV care including cotrimoxazole, isoniazid preventive therapy, screening for TB and cervical cancer, contraceptive advice, counseling and social support until they become eligible for ART. Following initiation of ART individuals needs to be supported within the same framework to ensure good adherence and retention in care.

We identified a number of important issues in our study. First, people who tested on their own initiative were least likely to have a timely CD4 count measurement done, underscoring the need to ensure that scale up of VCT programmes will be accompanied by clear plans to ensure that those who test positive go on to receive appropriate care. Second, almost a third (28%) of eligible patients with TB did not receive ART despite recommendations in favour of concomitant treatment since 2003 [17], and ART being associated with a 64–95% reduction in mortality in such patients [10,18,19,20]. This underscores the importance of integrating HIV and TB services [21].

Table 4. Factors associated with having a CD4 count ≤200 cells/μl within 6 months of HIV diagnosis.

Variables	ANC	STI	Tb	VCT
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Female	NA	1	1	1
Male	NA	0.82 (0.36–1.86)	0.97 (0.62–1.50)	1.27 (0.99–1.63)
Age<30 years	1	1	1	1
Age≥30 years	**2.42 (1.03–5.68)**	2.00 (0.92–4.35)	1.10 (0.68–1.78)	**1.40 (1.07–1.82)**
Tested in 2004–2006	1	NA	1	1
Tested in 2007–2009	1.32 (0.57–3.08)	NA	1.18 (0.75–1.85)	0.94 (0.74–1.19)

ANC = antenatal care, STI = sexual transmitted infections, TB = tuberculosis, VCT = voluntary counseling and testing.
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This study shows that men and younger adults fail to access health services efficiently. Only 30% of clients tested for HIV were men. This is consistent with studies showing that HIV-infected men are less likely to access treatment [22,23], present with more advanced stages of HIV disease [24] and have a higher mortality risk during ART [25,26,27,28,29,30,31,32]. Repeated CD4 counts were less likely in individuals under 30 years of age as also reported elsewhere[33].

It is important to note that less than half of patients whose first CD4 count was above the ART eligibility threshold came back for a repeat test. One way of improving ART uptake, and thus reduce mortality among patients who are otherwise lost to care, might be to change the CD4 threshold to 350 cell/μl in line with the latest World Health Organization recommendations [34].

Our overall finding that 33% of patients eligible for ART were lost to care is consistent with several reports from elsewhere in southern Africa. In a programme report from South Africa, only 55% of patients had a CD4 count measurement within 8 weeks of HIV diagnosis and 81% of eligible patients were on ART at 3 months follow-up [6,7]. Out of 2483 patients eligible for ART in Uganda 637 (26%) did not start ART; a third of these patients died before ART initiation and another quarter were alive but not taking ART [25]. In Mozambique only 57% of patients testing HIV positive entered HIV care and 31% of patients eligible for ART started ART within 3 months [35].

In our study only 63% of patients testing positive for HIV attended for a CD4 count measurement within 6 months. These outcomes are worse than those recently reported by a public-sector clinic in Johannesburg where 84.6% of patients who tested positive for HIV had a CD4 count measurement. The majority of these patients did not return for their CD4 result within 12 weeks [36]. Data from the same clinic in Johannesburg showed that among patients not yet eligible for ART only 26% returned for a scheduled pre-ART medical visit within one year compared to 43% of our patients not yet eligible for ART returning for a repeat CD4 count [37].

Substantial improvement in linkage to HIV care for TB and VCT patients was observed in more recent years in this study and yet this was not accompanied by improvements in linkage to ART. Failure of linkage to HIV and ART services translates into incomplete ART coverage at population level, seriously undermining the potential for reductions in mortality, morbidity, TB incidence and HIV transmission.

The study has several strengths and limitations. Strengths include that the study was conducted in a routine clinical program where CD4 count testing and ART were provided free. Thus, the results should be generalisable to similar settings. The study was conducted over a prolonged period with increasing ART availability. Among the limitations is the fact that patients might have been misclassified as failing to link to care if they accessed care with a service provider other than the primary health care clinic or hospital. Thus, linkage to care might be underestimated. However the nearest other ART site is more than 10 km away, and residents of this poor community are unlikely to have sought care in such a distant ART site unless they had moved away. Second, we did not assess if patients who had a CD4 count measurement actually returned to receive the result. Thirdly, we did not investigate reasons for not linking to care. Studies that have ascertained outcomes among patients lost to care have reported that up to a third of patients who failed to initiate ART had died [7,8,25]. Time cut-offs for linkage to care for both timely CD4 count and ART initiation are somewhat arbitrary. When no time cut-offs were used 75.3% (95% CI 70.3–80.3) of eligible individuals who had a CD4 count at some point during the study period eventually initiated ART.

In conclusion, while considerable attention has been paid to loss to follow-up and mortality among patients receiving ART [32,38,39,40], data on losses at earlier stages of the care pathway are scarce. As our study shows, a focus only on outcomes of those patients fortunate enough to initiate treatment fails to account for a substantial number of patients who are eligible for ART but do not receive it or not yet eligible but fail to reappear. Pre-ART defaulting should be encouraged in programme reporting. Programme adaptation to ensure retention in care between testing and ART should consider point of care CD4 count testing at time of HIV diagnosis as well as provision of integrated TB and HIV.

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Author Contributions

Analyzed the data: KK. Wrote the paper: KK. Designed the study and collected data: KK. Oversaw the field site and collected data, was involved in writing the paper: JZ. Oversaw the field site and collected data, contributed to and approved the final version of the paper: PG CO NK. Gave input on writing the paper, contributed to and approved the final version of the paper: SL. Responsible for the research infrastructure, contributed to and approved the final version of the paper: LGB. Responsible for the research infrastructure, gave input on writing the paper, contributed to and approved the final version of the paper: RW.

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When to initiate highly active antiretroviral therapy in sub-Saharan Africa? A South African cost-effectiveness study

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Background: Large-scale programmes increasing access to highly active antiretroviral therapy (HAART) are being implemented in sub-Saharan Africa. However, cost-effectiveness of initiating treatment at different CD4 count thresholds has not been explored in resource-poor settings.

Methods: A cost-effectiveness analysis was conducted from a public health perspective using primary treatment outcomes, healthcare utilisation and cost data (Jan 2004 local prices; US$1=7.6 Rands) derived from the Cape Town AIDS Cohort. A Markov state-transition model was developed to estimate life-expectancy, lifetime costs, quality-adjusted life-years (QALYs), cost per life-year and QALY gained for initiating HAART at three CD4 cell count thresholds (<200/μl, 200–350/μl and >350/μl), including the no antiretroviral therapy (No-ART) alternative. Each treatment option was compared with the next most effective undominated option.

Results: Mean life-expectancy was 6.2, 18.8, 21.0 and 23.3 years; discounted (8%) QALYs were 3.1, 6.2, 6.7 and 7.4; and discounted lifetime costs were US$5,250, US$5,434, US$5,740, US$6,588 for No-ART, and therapy initiation at <200/μl, 200–350/μl and >350/μl scenarios respectively. Clinical benefits increased significantly with early therapy initiation. Initiating therapy at <200/μl had an incremental cost-effectiveness ratio (ICER) of US$54 per QALY versus No-ART, 200–350/μl had an ICER of US$616 versus therapy initiation at <200/μl, and >350/μl had an ICER of US$1,137 versus therapy initiation at 200–350/μl. ICERs were sensitive to HAART cost.

Conclusions: HAART is reasonably cost-effective for HIV-infected patients in South Africa, and most effective if initiated when CD4 count >200/μl. Deferring treatment to <200/μl would reduce the aggregate cost of treatment, but this should be balanced against the significant clinical benefits associated with early therapy.

Introduction

The recognition of toxicities associated with long-term use of highly active antiretroviral therapy (HAART) resulted in a pendulum shift in expert opinion from an initial 'hit-early hit-hard' strategy to a more conservative strategy of deferring treatment to CD4 count <200 cells/μl [1,2]. However the case for earlier treatment is being re-examined [3]. Current guidelines recommend initiating therapy at a variety of CD4 cell count thresholds: <200/μl, <350/μl or >350/μl for patients with high plasma HIV RNA count [4–8]. The CD4 count threshold for therapy initiation is an important determinant of both clinical benefit and cost-effectiveness of HAART [1–3, 9–15], which is of particular relevance to sub-Saharan Africa, where resources are scarce and the burden of disease is large.

In sub-Saharan Africa the spectrum of opportunistic infections differs from that reported in industrialized countries [16–19]. *Mycobacterium avium* complex, cytomegalovirus infection and toxoplasmosis are less frequently diagnosed. Bacterial diseases are common and can occur at early stages of immune suppression [16–19]. Cryptococcal disease is a common cause of death [16,18], and tuberculosis, the most frequent opportunistic infection, is associated with accelerated course of HIV infection [20]. However, the rarity of

these diagnoses could be related to limited diagnostic resources or that patients die of commoner illnesses prior to onset of *Mycobacterium avium* complex or cytomegalovirus infection. Therefore, the clinical benefits of HAART and consequently the cost-effectiveness of therapy initiation demonstrated in industrialized countries may not be generalizable to populations in sub-Saharan Africa. In this study, we assessed the impact of initiating therapy at CD4 >350/μl; 200–350/μl or <200/μl on healthcare utilization, lifetime costs, life-expectancy and the quality of life of adult HIV-infected patients.

Methods

Study sample

This analysis was based on the Cape Town AIDS Cohort study (CTAC). Methods of recruiting patients to the cohort are described elsewhere [21]. In brief, CTAC was one of the largest sub-Saharan African HIV cohorts, and is composed of patients referred from a wide range of primary health care facilities in Cape Town to the adult HIV clinics affiliated to the University of Cape Town from 1992 to 2005. Patients of this cohort are likely to represent urban populations that would seek care in the proposed rollout of antiretroviral therapy (ART) in South Africa. During the period of this study, ART was not available in the public sector, and CTAC patients accessed ART through international multicentre Phase III clinical trials [22–26], which were conducted between 1996 and 2005, and were approved by the University of Cape Town Research Ethics Committee.

The objectives of this study were to estimate utilization and cost of HIV healthcare, and to assess the cost-effectiveness of initiating HAART at different CD4 cell count thresholds and a no antiretroviral therapy alternative (No-ART). The first objective provides primary input data for the second, a modelled cost-effectiveness analysis.

Healthcare utilization and cost

Costing was undertaken from a public health systems perspective. Healthcare utilization was extracted from hospital records. The mean number (and 95% CI) of inpatient days and outpatient visits per patient-year was calculated for each CD4 count category. Unit costs were obtained from a costing study of HIV healthcare conducted locally in 2000 [27]. In this study, costs relating to usage of medicines, including treatment and prophylaxis for opportunistic infections and other non-ART drugs dispensed to the patients during the course of care, and medical tests and procedures were assessed by extracting data from outpatient and inpatient medical records using the micro-costing methods [28]. Consultancy, doctor, nursing and capital costs were allocated using step-down accounting methods [29]. These costs were adjusted to 2004 prices using the South African Consumer Price Index, excluding mortgage bonds [30]. Hospital overheads and hotel costs were recalculated from 2003/2004 expenditure data, again using step-down accounting methods. Estimates were converted to US dollars (US$1=7.6 Rands) [31]. The cost per inpatient day and outpatient visit was US$182 and US$28 respectively, which is similar to estimates from another analysis conducted in South Africa [32].

A variety of ART drugs were used in the clinical trials. HAART was defined as a non-nucleoside reverse transcriptase inhibitor or protease inhibitor together with two nucleoside analogues or three nucleoside analogues. However, we considered costs for two ART regimens currently recommended in the South African public sector [33]: first-line regimen of stavudine, lamivudine and efavirenz or nevirapine, and for those virologically failing, a second-line regimen of zidovudine, didanosine and lopinavir/ritonavir. The relative proportion of patients on efavirenz versus nevirapine was derived from a community pilot project [32]. ART drugs costs were based on the official public sector tender prices [Channing L, personal communication, 2004]. Viral load test was measured every 6 months if viral load were <400 or between 400–5,000 copies/μl, or every 3 months if >5,000 copies/μl, as per national guidelines. Viral load costs were obtained from the National Health Laboratory Service.

Cost-effectiveness analysis

Model description. Cost-effectiveness analyses were conducted using a Monte Carlo simulated Markov state-transition model. The model included seven health states defined by three CD4 cell count categories (<200/μl, 200–350/μl and >350/μl), which were further stratified according to WHO Non-Aids (WHO stage 1, 2 or 3) and AIDS (WHO stage 4) clinical stages, and death. The treeAge Software (Williamstown, MA, USA) was used to develop the model. To increase the precision of estimates, a cohort of 10,000 patients with sociodemographic characteristics similar to CTAC patients (Table 1) was followed through the model over a sequence of 1 month cycles. The model was run over a 50 year period, such that greater than 95% of the cohort was dead when the run of the model terminated. The model structure is presented in Figure 1.

In the HAART alternative, the effectiveness of treatment was modelled as a rate of progression to higher CD4 cell count strata, whereas the outcome of

Table 1. Baseline demographic and clinical characteristics of the highly active antiretroviral therapy group (*n*=332) and the no-antiretroviral therapy group (*n*=1284)

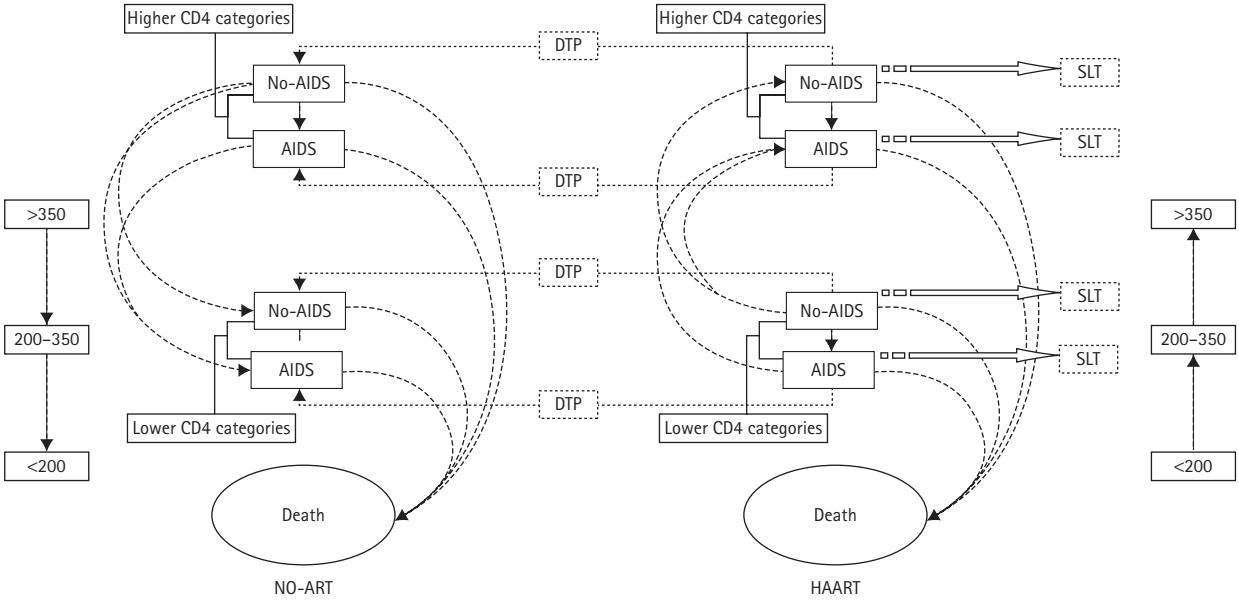
Characteristic	HAART *n* (%)	No-ART *n* (%)	*P*-value*
Age			0.60
<32 years	151 (45.5)	672 (52.3)	
≥32 years	147 (54.5)	612 (47.7)	
Gender			0.03
Male	182 (54.8)	616 (48)	
Female	150 (45.2)	668 (52)	
Socioeconomic status			<0.0001
High	161 (45.5)	428 (33.3)	
Low	171 (51.5)	856 (66.7)	
WHO stage			0.12
Stage 1 & 2	169 (50.9)	667 (51.9)	
Stage 3	121 (36.5)	407 (31.7)	
Stage 4	42 (12.6)	210 (16.4)	
CD4 cell count			0.003
<200/μL	126 (38)	529 (41.2)	
200–350/μl	116 (35)	331 (25.8)	
>350/μl	90 (27)	424 (33)	
Viral load			
Mean (log_{10} copies/μl)	5.0	-	

*χ^2 test. HAART, highly active antiretroviral therapy; NO-ART, no-antiretroviral therapy.

the No-ART alternative was modelled as a rate of progression to a lower CD4 cell count strata, taking into account the clinical WHO stage of each patient, with death as the absorbing state in both alternatives. Progression rates were calculated as the number of events occurring over a 1 month period and were expressed as transition probabilities using a binomial distribution. The model incorporated all possible pathways of moving from one CD4 strata to another, including the transition probabilities of switching from first-line to second-line HAART therapy, or discontinuing therapy due to toxicity, withdrawal of consent or loss to follow-up. Other trial-related reasons for stopping HAART, such as pregnancy were excluded from the probability of discontinuing therapy. Non-HIV related mortality was captured using the South African life-tables [34], adjusted for age, gender and socioeconomic status. Patients with an AIDS diagnosis were assumed to begin therapy immediately irrespective of CD4 level.

The model assigned healthcare utilization, cost estimates and quality of life weights to the respective health states. The cost-effectiveness ratio was calculated as the incremental cost per life year and per quality-adjusted life-year (QALY) gained, comparing each option with the next most effective, after ruling out options with higher cost and lower effectiveness

Figure 1. A state transition diagram representing the CD4 cell count and WHO clinical stage based Markov model for No-antiretroval therapy and highly active antiretroviral therapy states



Patients dropping out of the highly active antiretroviral therapy (HAART) group will have the same transition probability as the respective no-antiretroviral therapy (No-ART) group from the date they stop treatment (DTP). HAART patients discontinuing first-line therapy (for reasons other than toxicity, with drawing consent or loss to follow-up) will switch to second line therapy (SLT; see Table 3).

(simple dominance) or those with higher incremental cost-effectiveness ratios (ICER) relative to more effective options (extended dominance), as recommended by the USA Public Health Service Expert Panel on Cost-effectiveness in Health and Medicine [35]. Future costs and benefits were discounted at an annual rate of 8%, which is widely used in South Africa, and is based on the return on long-term government bonds [36].

Health-related quality of life data. Health-related quality of life data were derived from two local studies. The first assessed the impact of HIV infection on quality of life [37], and the second evaluated changes in quality of life attributable to HAART [38]. Both studies used the Medical Outcomes Survey Short Form (SF-36). These data were converted to SF-6D; a simplified health state classification based on selected items from SF-36. SF-6D health states were converted into QALYs using United Kingdom general population standard gamble valuations from a survey undertaken by Brazier *et al.* [39].

Sensitivity analysis. The robustness of results were assessed in probabilistic and one-way sensitivity analyses. The probabilistic analyses were undertaken to reflect, in the results of the model, the uncertainty associated with parameters, as recommended by the National Institute for Clinical Excellence [40]. This involved constructing triangular distribution based on 95% CI for healthcare utilization, and binomial distributions for transition probabilities. The impact of these parameter uncertainties was propagated through the model by means of second-order Monte Carlo simulation. This involved running the model 10,000 times using randomly selected values from input distributions in order to derive probability distributions for model outputs [41]. One-way sensitivity analyses included a no second-line HAART scenario, ART price reductions, reduced No-ART healthcare costs, and 0% and 3% discounting of costs and benefits. Further uncertainties typical to HAART cost-effectiveness analyses, including overall durability of ART regimens, costs and outcomes after treatment failure [9–11] were also examined. In addition, we have extensively explored whether differential efficacy would impact on our cost-effectiveness rankings. This was done by increasing the probability of failing firs line,and increasing the probability of dying and discontinuing therapy while on second-line.

Results

Study sample

The demographic and baseline clinical characteristics of the study cohort (332 HAART and 1,284 No-ART patients) are described in Table 1. In the HAART group, 48 patients (incidence density rate [IDR]=2.59 per 100 patient-years [PYS]; 95%CI 1.91–3.44) discontinued treatment permanently due to toxicity, withdrawal of consent or loss to follow up, and 51 patients (IDR=2.76 per 100 PYS; 95%CI 2.05–3.63) switched to second-line or salvage therapy. In the No-ART group 295 patients died compared with 34 patients in the HAART group. Death events occured in HAART patients with CD4 cell count of >350/μl (IDR=0.40 per 100 PYS, 95%CI 0.08–1.18) or 200-350/μl (IDR=1.18 per 100 PYS, 95%CI 0.38–2.75), were significantly less than in those with <200/μl (IDR=3.94 per 100 PYS, 95%CI 2.57–5.77); *P*-value <0.05 and <0.001 respectively. The transition probabilities from one health state to another for the two groups are summarized in Table 2.

Healthcare utilization and cost

Across all health states, inpatient days were significantly lower in the HAART group compared with the No-ART group. Inpatient and outpatient use was higher in patients with advanced immune suppression and clinical disease (Table 3). The annual cost of first-line and second-line regimens was US$322 and US$840 respectively, and monitoring viral load cost was US$79. The monthly total cost of healthcare increased from US$463 in No-ART patients with CD4 cell counts >350/μl and no AIDS to US$3,434 in those with <200/μl and AIDS. A similar trend was observed in HAART patients (Table 3).

Cost-effectiveness analysis

The undiscounted mean life-expectancy was 6.2 years for No-ART patients compared with 18.8, 21 and 23.3 years for HAART patients initiating therapy with CD4 cell count of <200/μl, 200–350/μl and 351–500/μl respectively. Similar trends were observed for QALYs (Table 4).

The No-ART discounted QALYs were 3.1, 6.2, 6.7 and 7.4 for No-ART and patients starting HAART at CD4 cell count <200/μl, 200–350/μl and >350/μl respectively. Initiating therapy at <200/μl had an ICER of US$54 per QALY versus No-ART, initiating therapy at 200–350/μl had an ICER of US$616 versus <200/μl, and >350/μl had an ICER of US$1,137 versus therapy initiation at 200–350/μl. ICERs were sensitive to HAART cost. Compared with other therapy initiation scenarios, starting with >350/μl was the most effective option but the most costly, owing to higher projected life expectancy (Table 4).

Sensitivity analysis

Confidence intervals presented in Table 4 indicate the impact of parameter uncertainty on cost-effectiveness results (propagated through the model via probabilistic

Table 2. Monthly health states transition probabilities of the highly active antiretoviral therapy and no-antiretroviral therapy groups

	From state		To state						
				<200/μl		200–350/μl		>350/μl	
			Death	No-AIDS	AIDS	No-AIDS	AIDS	No-AIDS	AIDS
No-ART	<200/μl	No-AIDS	0.01265	-	0.02598	-	-	-	-
			0.0099–0.0159	-	0.0222–0.0303	-	-	-	-
		AIDS	0.05156	-	-	-	-	-	-
			0.0444–0.0596	-	-	-	-	-	-
	200–350/μl	No-AIDS	0.00391	0.0227	0.009	-	0.0051	-	-
			0.0023–0.0062	0.0186–0.02737	0.0067–0.0119	-	0.0033–0.0076	-	-
		AIDS	0.009488	-	0.035	-	-	-	-
			0.0041–0.0187	-	0.0233–0.0514	-	-	-	-
	>350/μl	No-AIDS	0.001262	0.0041	0.0055	0.01444	0.00063	-	0.0018
			0.0006–0.0023	0.0028–0.0058	0.0040–0.0074	0.0119–0.0174	0.0003–0.0015	-	0.0010–0.0031
		AIDS	0.002225	-	-	-	0.03119	-	0.0183
			0.00007–0.0124	-	-	-	0.0156–0.0558	-	0.0084–0.0348
HAART	<200/μl	No-AIDS	0.0018	-	0.0026	0.02739	0.00127	0.02285	0.00089
			0.0001–0.0032	-	0.0015–0.004	0.0194–0.0289	0.0006–0.0024	0.0193–0.027	0.0003–0.0.0019
		AIDS	0.01129	-	-	-	0.1018	-	0.02215
			0.0062–0.0189	-	-	-	0.0788–0.1295	-	0.014–0.033
	200–350/μl	No-AIDS	0.00089	-	-	-	0.00155	0.07588	0.00089
			0.0002–0.0023	-	-	-	0.0006–0.0032	0.0648–0.0888	0.0002–0.0023
		AIDS	0.00166	-	-	-	-	-	0.00988
			0.00004–0.00923	-	-	-	-	-	0.0027–0.0253
	>350/μl	No-AIDS	0.00024	-	-	-	-	-	0.00049
			0.00003–0.00087	-	-	-	-	-	0.0001–0.0012
		AIDS	0.00155	-	-	-	-	-	-
			0.00004–0.0086	-	-	-	-	-	-

HAART, highly active antiretroviral therapy; No-ART, no-antiretroviral therapy.

Table 3. Annual care utilization and cost estimates of the highly active antiretroviral therapy and no-antiretroviral therapy groups by health states

	Inpatient day (cost per day=US$182)				Outpatient visits (cost per visit=US$28)				Total cost (95%CI)		
	No-ART		HAART		No-ART		HAART		No-ART	HAART	
Health state	Mean IP days (95%CI)	Cost (95%CI)	Mean IP days (95%CI)	Cost (95%CI)	Mean OP visits (95%CI)	Cost (95%CI)	Mean OP visits (95%CI)	Cost (95%CI)		First line	Second line
CD4<200 cells/μl											
No-AIDS	7.7	1,397	0.26	47	5.6	157	3.9	109	1,555	558	1,075
	7.4–7.9	1,352–1,444	0.22–0.31	40–56	5.4–5.8	151–163	3.7–4.1	105–114	1,503–1,607	546–571	1,063–1,088
AIDS	17.7	3,218	1.8	359	7.7	215	5.6	156	3,434	916	1,433
	17.2–18.1	3,132–3,298	1.7–2.2	315–407	7.4–8.0	207–225	5.1–6.0	144–168	338–3,522	860–976	1,378–1,494
CD4 200–350 cells/μl											
No-AIDS	3.0	545	0.39	71	5.1	142	3.9	110	687	582	1,099
	2.8–3.2	514–577	0.33–0.46	60–83	4.9–5.3	136–148	3.7–4.1	104–115	650–726	565–600	1,083–1,117
AIDS	10.8	1971	0.52	94	4.9	138	3.8	106	2,109	602	1,119
	10.1–11.6	1,835–2,114	0.34–0.76	61–138	4.4–5.5	124–153	3.3–4.4	92–122	1,960–2,267	555–662	1,072–1,179
CD4>350cells/μl											
No-AIDS	1.9	348	0.14	25	4.1	114	4.6	129	463	555	1,073
	1.8–2.0	329–368	0.11–0.17	20–31	3.9–4.2	110–119	4.4–4.8	124–133	439–487	546–565	1,063–1,083
AIDS	5.7	1,035	0.37	67	4.2	117	3.9	108	1,153	577	1,094
	4.9–6.5	902–1,183	0.23–0.57	41–104	3.6–4.9	100–137	3.4–4.4	94–124	1,002–1,320	536–629	1,054–1,147

First-line highly active antoretroviral therapy (HAART; cost per month=US$26.86): stavudine, lamivudine and efavirenz (mean of 61% of patients on first-line therapy) or nevirapine (mean of 39% of patients on first-line therapy). Second-line HAART (cost per month=US$70.00): zidovudine, didanosine and lopinavir/ritonavir. Total cost of first-line and second-line therapy include viral load monitoring (cost per month=US$6.58). IP, inpatient; OP, outpatient.

Table 4. Cost-effectiveness of different treatment options for HIV-infected patients

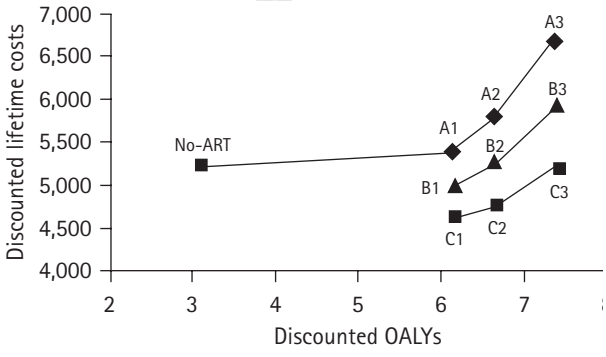
Treatment option	Total lifetime costs	Effectiveness		ICER (incremental cost/incremental effect)	
		Life years	QALY	Life years	QALY
Discounted (8%)					
No-ART	5,250 (4,953–5,577)	4.5 (4.3–4.7)	3.1 (3.0–3.3)	N/A	N/A
CD4 <200 cells/μl	5,434 (4,976–5,907)	8.2 (7.6–8.7)	6.2 (5.8–6.7)	46 (-240–166)	54 (-115–197)
CD4 200–350 cells/μl	5,740 (5,365–6,122)	8.7 (8.2–9.2)	6.7 (6.3–7.1)	577 (440–668)	616 (444–733)
CD4 >350 cells/μl	6,588 (6,272–6,893)	9.3 (8.9–9.7)	7.4 (7.1–7.8)	1,263 (1,069–2,209)	1,137 (1,011–1,317)
Undiscounted					
No-ART	7,877 (7,315–8,472)	6.2 (5.8–6.6)	4.3 (4.0–4.6)	N/A	N/A
CD4 <200 cells/μl	14,230 (11,984–16,817)	18.8 (16.3–21.7)	14.6 (12.5–16.9)	499 (405–583)	611 (491–719)
CD4 200–350 cells/μl	15,880 (13,927–18,005)	21.0 (18.7–23.4)	16.4 (14.5–18.4)	766 (676–829)	915 (787–996)
CD4 >350 cells/μl	18,505 (16,745–20,347)	23.3 (21.2–25.6)	18.5 (16.8–20.4)	1,148 (971–1,635)	1,236 (1,110–1,468)

Costs and effects are per patient. ICER, incremental cost-effective ratio; N/A, not applicable; No-ART, no anti retroviral therapy; QALY, quality adjusted life years.

sensitivity analysis). One-way sensitivity analyses indicated that results were not sensitive to cost and outcome after treatment failure, to cost reductions in No-ART health states, to discount rate variations, to increased failure rates of first-line therapy and to reduced efficacy of second-line therapy.

Results were sensitive to HAART price. Figure 2 shows the impact of decreased HAART prices on the cost-effectiveness of therapy. At 40% price reduction, HAART therapy initiation will be cost-saving relative to No-ART at all CD4 cell count thresholds (Figure 2). Similarly, initiating therapy at <200/μl and 200–350/μl was cost-saving relative to No-ART if patients were assumed to receive first-line therapy only.

Figure 2. The impact of HAART price on the cost-effectiveness of different strategies of iniating HAART



A-C represent 0%, 20% and 40% highly active antiretroviral therapy (HAART) price reductions respectively. 1–3 represent CD4 cell count of <200/μl, 200–350/μl and >350μl respevtively. Points lying below no-antiretroviral therapy (No-ART) indicate cost saving options (absolute dominance). Points above No-ART indicate more effective but more costly options of HAART; the incremental cost effectiveness ratio is represented by the slope of the lines joining the points. QALY, quality-adjusted life year.

Discussion

This is the first study from sub-Saharan Africa to assess the cost-effectiveness of initiating HAART at different CD4 cell count thresholds in ART-naive individuals. Hospitalization increased with advancing immune suppression, but was significantly reduced in patients receiving HAART. Due to higher life-expectancy, total lifetime costs in the HAART group exceeded that of the No-ART alternative. Initiating therapy at 200–350/μl and above 350/μl was more effective, but requires more resources than deferring therapy to CD4 cell count <200/μl.

Due to paucity of studies, cost-effectiveness has not been routinely used in South Africa to inform public health resource allocation policy, and it is not possible to compare the ICERs in this study with other healthcare interventions. However, the Commission on Macroeconomics and Health has suggested that interventions that have an ICER that is less than per capita gross domestic product (US$ 3,089 in 2003 in South Africa) could be considered to be very cost-effective [42]. Since the ICER estimates in this study are much lower than this threshold, all therapy initiation scenarios could be considered cost-effective by this criterion.

Our findings concur with reports from industrialized countries where initiation of therapy at CD4 cell counts higher than 200/μl impacted favourably on clinical progression [2,10,13,14,15,43]. A recent review concluded that earlier initiation of HAART could be associated with better immune improvement, less drug-related toxicity, and reduced HIV transmission [2]. In a study with extended follow-up, risk of disease progression was increased, even with durable virological suppression, when initiating HAART at CD4 cell counts <200/μl, compared with 200–350/μl or >350/μl

[43]. In previous analyses performed in our cohort, early therapy initiation was associated with reduced incidence of tuberculosis [21], AIDS and death compared with deferring treatment to <200/μl [44]. Life-expectancy on HAART in this study ranged between 18.8 and 23.3 years compared with 31.1 years reported in patients of similar age in a large European HIV cohort study [45]. Both studies involved extrapolation beyond follow-up, without restricting efficacy of treatment as conservatively assumed in other studies [9–11].

This study has several limitations. The treated arm included participants in clinical trials. Management of these patients may not reflect the standard or intensity of care provided for patients not participating in clinical trials. In addition, patients participating in the HAART clinical trials were not randomly select from the whole cohort, but were those patients who met inclusion criteria. Due to the limited availability of drugs in these trials, patients were invited to participate on a first-come, first-served basis. Therefore, it is difficult to exclude the possibility of some selection bias in recruiting patients into the clinical trial or to ensure that characteristics of patients in the two groups studied were strictly similar. However, outcomes for these patients were not dissimilar from those reported in large developed country cohort analyses [46]. The design of our model also accounted for the most measurable clinical and immunological confounding factors. Starting therapy at a wider range of CD4 cell counts in this cohort than is recommended, in the South African ART rollout programme, allowed us to assess the cost-effectiveness of the initiation of therapy under different scenarios. Because the sample size of the treated group was relatively small, we used one-way and probabilistic sensitivity analysis of 10,000 simulated patients to approximate lifetime estimates, and to ensure that uncertainty relating to data sources and methodological choices was accounted for.

Healthcare cost estimates in this study are comparable to a recent community-based South African study by Cleary *et al.* [32], but higher than reported in other African countries [47]. Viral load measurement was not available for the No-ART group therefore we were unable to adjust for the level of HIV RNA on disease progression. However, it has been shown that CD4 cell count is a better predictor of disease progression than viral load [12]. We did not assess the cost implications of our findings for the South African HAART rollout programme because at present no data exist on the distribution of HIV-infected patients by CD4 cell count for the country. This study was conducted from a public healthcare perspective. The societal perspective would capture added indirect costs or benefits associated with HAART, such as productivity gains and opportunity costs associated with seeking care, which could potentially further improve the cost-effectiveness of HAART [11]. However, valuing productivity gains in a largely unemployed population is fraught with ethical problems [48]. Furthermore, these data do not currently exist. Costs of the No-ART option reflect a level of urban access to healthcare that may not be available in remote areas of South Africa. However, HAART initiation ICERs were not sensitive to reductions in No-ART costs, and 'do-nothing' was not considered a reasonable policy alternative. Moreover, the rollout is intended to start from settings where infrastructure currently exists, which would predominantly be urban.

Our findings indicate that HAART is a cost-effective public health intervention in South Africa. The decision of when to start therapy is complex and should carefully balance reducing costs while maximizing the population benefits of treatment within existing operational limitations. The aggregate cost of starting HAART at CD4 cell counts >200/μl may be higher than deferring treatment to <200/μl, but due to the high rates of untreated disease progression at CD4 cells above 200/μl in sub-Saharan Africa, countries with less binding resource constraints should consider earlier therapy initiation to take advantage of additional clinical benefits.

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CD4 cell count recovery among HIV-infected patients with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa

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Abstract

Background: Patients accessing antiretroviral treatment (ART) programmes in sub-Saharan Africa frequently have very advanced immunodeficiency. Previous data suggest that such patients may have diminished capacity for CD4 cell count recovery.

Methods: Rates of CD4 cell increase were determined over 48 weeks among ART-naïve individuals (n = 596) commencing ART in a South African community-based ART programme.

Results: The CD4 cell count increased from a median of 97 cells/μl at baseline to 261 cells/μl at 48 weeks and the proportion of patients with a CD4 cell count <100 cells/μl decreased from 51% at baseline to just 4% at 48 weeks. A rapid first phase of recovery (0–16 weeks, median rate = 25.5 cells/μl/month) was followed by a slower second phase (16–48 weeks, median rate = 7.7 cells/μl/month). Compared to patients with higher baseline counts, multivariate analysis showed that those with baseline CD4 counts <50 cells/μl had similar rates of phase 1 CD4 cell recovery (P = 0.42), greater rates of phase 2 recovery (P = 0.007) and a lower risk of immunological non-response (P = 0.016). Among those that achieved a CD4 cell count >500 cells/μl at 48 weeks, 19% had baseline CD4 cell counts <50 cells/μl. However, the proportion of these patients that attained a CD4 count 200 cells/μl at 48 weeks was lower than those with higher baseline CD4 cell counts.

Conclusion: Patients in this cohort with baseline CD4 cell counts <50 cells/μl have equivalent or greater capacity for immunological recovery during 48 weeks of ART compared to those with higher baseline CD4 cell counts. However, their CD4 counts remain <200 cells/μl for a longer period, potentially increasing their risk of morbidity and mortality in the first year of ART.

Background

The World Health Organisation (WHO) estimated that in June 2005 4.7 million people living in sub-Saharan Africa were in urgent need of antiretroviral treatment (ART) [1]. Despite formidable logistical hurdles, the number of individuals able to access this treatment in the region is

expanding. One of the programmatic challenges facing ART services in sub-Saharan Africa is that many HIV-infected patients only access healthcare once they have developed advanced symptomatic disease [2] and this delay may be further compounded by health-systems delays. The median CD4 cell count among those enrolling in ART programmes is often under 100 cells/μl even where programmes have been well established for several years [3-5].

Patients enrolling into ART programmes with very low CD4 cell counts have heightened risk of morbidity and mortality both before and during the initial months of ART [5-8]. Moreover, advanced pre-treatment immunodeficiency is also reported to be associated with diminished capacity for restoration of CD4 cell counts and CD4 cell functional responses during ART [9-14]. This raises the concern that many patients entering ART programmes in sub-Saharan Africa may have limited potential for immune recovery. Although previous studies from sub-Saharan Africa have reported overall CD4 cell count responses [3,15,16], there are no published data from the region regarding rates of CD4 cell recovery and rates of immunological non-response to ART among patients with CD4 cell <50 cells/μl.

In this study we have examined determinants of CD4 cell count recovery among patients accessing a community-based antiretroviral programme in South Africa. We focus on the hypothesis that advanced pre-treatment immunodeficiency diminishes the capacity for CD4 cell count recovery during ART as determined by rates of CD4 cell count increase and risk of immunological non-response.

Methods

Study population

We studied patients accessing ART at the Gugulethu Community Health Centre, Cape Town, South Africa [5]. The vast majority of patients receiving treatment at this clinic live in conditions of low socioeconomic status and HIV transmission is predominantly heterosexual. The South African national ART programme guidelines are based on the World Health Organisation (WHO) 2002 recommendations [17], with criteria for ART including those with a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count <200 cells/μl.

The first-line ART regimen was comprised of stavudine, lamivudine plus a non-nucleoside reverse transcriptase inhibitor (efavirenz or nevirapine). The second-line regimen for those failing first-line treatment was comprised of lopinavir/ritonavir, zidovudine and didanosine. Treatment adherence exceeds 90% at one year [18]. All treatment was free of charge and there were no interruptions in drug supply. All patients with CD4 counts <200 cells/μl received daily cotrimoxazole prophylaxis; dapsone was used as an alternative.

Data collection

Plasma HIV-1 load was measured at baseline and 4 monthly during treatment using Versant™ HIV-1 RNA 3.0 branched chain DNA assay (Bayer HealthCare, Leverkusen, Germany) with a lower limit of detection of 50 RNA copies/ml. Blood CD4 cell counts were measured at the same time-points by flow cytometry using FACS-Count™ (Becton Dickinson Inc., Franklin Lakes, NJ, USA). These assays were all performed in a single nationally accredited laboratory which has rigorous quality assurance procedures.

Structured clinical and laboratory records were maintained on all patients screened on entry to the ART programme and this information was transferred on a weekly basis to a computer database. Data were analysed from the start of the programme in September 2002 until data censorship in August 2005. All treatment-naive patients aged over 15 years and with at least a 16-week follow-up time point were included in the analysis. Study of these patients was in compliance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University of Cape Town; all patients enrolled gave written informed consent.

Data analysis

Data were analysed using Stata version 9.0 (College Station, Texas, USA). We calculated absolute responses in CD4 cell counts during three intervals (from baseline to 16 weeks of ART, 16 to 32 weeks, and 32 to 48 weeks), as well as rates of CD4 cell increase (cells/μl/month) during each interval. The first interval was used as an estimate of the initial (phase 1) response to ART. The CD4 cell count responses observed during the latter two intervals (16 to 32 and 32 to 48 weeks after treatment initiation) were very similar, and were combined into a single measure of the second phase of CD4 cell responses (phase 2).

We further evaluated CD4 cell responses in the following ways: i) whether patients failed to attain an absolute CD4 cell count increase from baseline of at least 50 cells/μl at 48 weeks (defined as immunological non-response); ii) whether patients achieved an absolute CD4 count of 200 cells/μl at the 48 week visit; iii) and whether patients had achieved an absolute CD4 cell count of 500 cells/μl at 48 weeks (super-responders).

In bivariate analyses, medians were compared using Wilcoxon rank-sum and sign-rank tests; proportions were compared using chi-square tests. All statistical tests were two-sided at alpha = 0.05. Separate multiple linear regression models were used to examine factors associated with

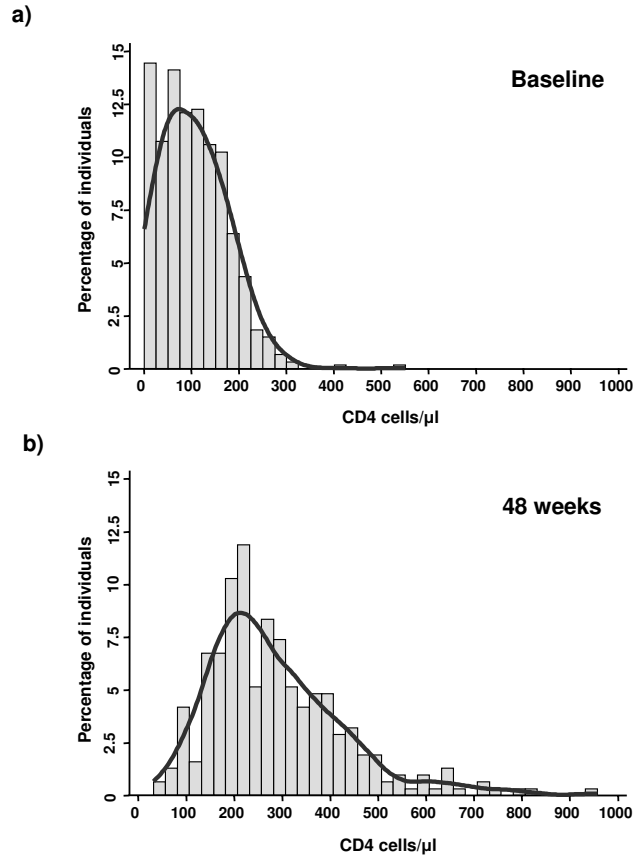


Figure 1
Graphs showing the frequency distribution of absolute blood CD4 cell counts (a) at baseline (n = 596) and (b) after 48 weeks (n = 311) of antiretroviral treatment. Smoothed curves have been fitted.

rates of CD4 cell count increase per month during the first and second phases. Baseline CD4 cell counts were categorised as follows: <50, 50–99, 100–149 and >150 cells/μl. Multiple logistic regression was used to assess factors associated with the binary outcomes of a CD4 cell increase of ≥ 50 cells/μl and ≥ 100 cells/μl during the 48 weeks of ART, as well as achieving an absolute CD4 cell count of ≥ 200 cells/μl during follow-up. Variables were included in multivariate models if they demonstrated a persistent association with the outcome of interest, or if their removal affected appreciably associations involving other variables [19].

Results

Baseline characteristics and follow-up

Of 698 individuals who commenced ART between September 2002 and April 2005, 596 (85%) had completed a 16-week follow-up visit at the time of data censorship, 34 (5%) were awaiting this appointment, 48 (7%) had died and 20 (3%) were either transferred out or were lost to follow-up. Of the 596 individuals who met the inclusion criteria for the study, 580 (97%) remained within the programme at study censorship, 11 (2%) died and 7 (1%) were lost from the programme.

At baseline the median age was 32 years (IQR, 28–38) and 75% were female. The median plasma viral load was 4.88 $\log_{10}$ RNA copies/ml (IQR, 4.50–5.27) and 58% of patients had a viral load >100,000 copies/ml. The median blood CD4 cell count was 97 cells/μl (IQR, 50–153) and the proportions of patients with CD4 cell counts within the ranges <50, 50–99, 100–149 and ≥ 150 cells/μl were 25%, 26%, 23% and 26%, respectively. Eighty per cent of patients had symptomatic disease, with 53% and 27% of patients having WHO stages 3 and 4 disease, respectively. During follow-up, only 7 (1%) patients switched to the second-line drug regimen.

Virological and CD4 cell responses to ART

Virological responses to treatment were excellent with viral load suppressed <400 copies/ml in ≥ 94% of patients at each of the follow-up time-points (Table 1). As a result, the frequency distribution of blood CD4 cell counts within the cohort shifted markedly from baseline over 48 weeks of treatment (Fig. 1). Whereas the proportion of individuals with CD4 cell counts <100 cells/μl was 51% at baseline, this proportion decreased to only 4% by week 48 of treatment. The median CD4 cell count increased almost 3-fold from baseline, reaching 261 cells/μl after 48 weeks ART (Table 1).

The rate of CD4 cell count increase in the first 16 week period greatly exceeded the rates in both the 16–32 week and 32–48 week intervals, but the rates did not differ comparing the latter two intervals. Thus, the pattern of CD4 cell count increase was divided into 2 phases: a rapid phase 1 (0–16 weeks; median = 25.5 cells/μl/month) and a slower phase 2 (16–48 weeks; median = 7.7 cells/μl/month).

Baseline characteristics and rates of CD4 cell count change in phase 1 and phase 2 did not differ when comparing the results of analyses of all eligible patients with those restricted to subjects who had data for every time-point (n = 292); this was also the case for all subsequent stratified analyses. Use of data from the larger cohort (n = 596) was therefore validated.

Effect of baseline CD4 cell count on rates of CD4 cell increases

Rates of phase 1 CD4 cell increase were similar across all CD4 cell count strata (Figure 2). Subsequent multivariate analysis found that baseline plasma viral load was the single factor independently associated with phase 1 CD4 cell recovery (Table 2). This association was strong; among

Table 1: Changes in blood CD4 cell counts and plasma viral load during ART.

	Baseline	Week 16	Week 32	Week 48
No. of patients	596	596	404	311
Virological Response				
Median VL	75,858	<50	<50	<50
Number (%) patients wilh VL ≥ 400	585 (98)	31 (5)	26 (6)	18(6)
Number (%) patients with VL ≥ 50	591 (99)	117 (20)	74 (18)	54 (17)
CD4 Cell Count Response				
Median (IQR) CD4 cell count (cells/μl)	97 (50–153)	199 (140,314)	226 (162, 314)	261 (193, 363)
Median (IQR) CD4 cell slope in preceding interval (cells/μl/month)	-	25.5 (12.7, 42.9)	7.5 (-4.6, 19.8)	7.9 (-3.0, 20.0)

VL = viral load (copies/ml)

those with a baseline viral load <5 $\log_{10}$ copies/ml the phase 1 CD4 cell slope was 21.3 cells/μl/month compared to 31.0 cells/μl/month among those with baseline viral load of >5 $\log_{10}$ copies/ml (P < 0.001).

Contrary to our initial hypothesis, the rate of phase 2 CD4 cell increase was greater among those with a baseline CD4 cell count <50 cells/μl compared to those with higher baseline counts (Figure 2). This association was highly significant in multivariate analysis (Table 2). The rate of phase 2 CD4 cell increase was also greater among younger compared to older patients (Figure 3). Multivariate analysis confirmed that both low baseline CD4 cell count and younger age were the only baseline characteristics that were significantly associated with higher rates of phase 2 CD4 cell recovery (Table 2).

In the multivariate model predicting rates of phase 2 CD4 cell increase, factors associated with the response to ART during the first 16 weeks were also included. A lower rate of phase 1 CD4 cell increase and full suppression of viral load at 16 weeks were both strongly associated with greater rate of phase 2 CD4 cell increase. Viral load suppression <400 copies/ml at 16 weeks was associated with a subsequent rate of CD4 cell increase of 6.8 cells/μl/month compared to 0.7 cells/μl/month among those whose viral load remained >400 copies/ml (P < 0.001).

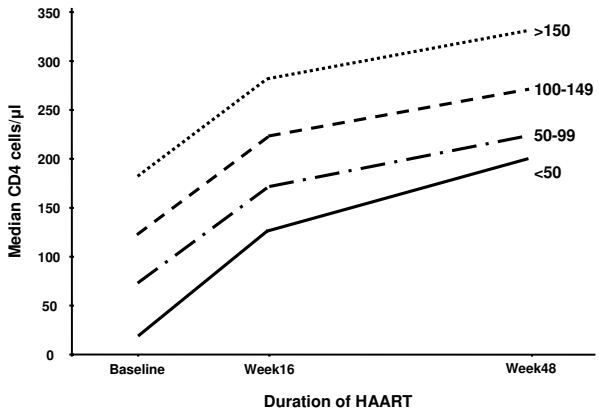
Baseline CD4 cell count and risk of immunological non-response

We next examined whether baseline CD4 cell count was a risk factor associated with immunological non-response (defined as a CD4 cell increase of <50 cells/μl at 48 weeks). Among those followed to 48 weeks (n = 311), the blood CD4 cell count increased by <50 cells/μl among 30 (9.7%) patients; of these, the viral load was suppressed <50 copies/ml among 22 patients, representing a treatment discordance rate of 7%. Contrary to our initial hypothesis, low baseline CD4 cell counts were not associated with increased risk of immunological non-response.

Table 2: Multiple linear regression models estimating the average change in CD4 cell count per month (CD4 slope). Separate models analyse the first (0 – 16 weeks) and second (16 – 48 weeks) phases of immune recovery during ART.

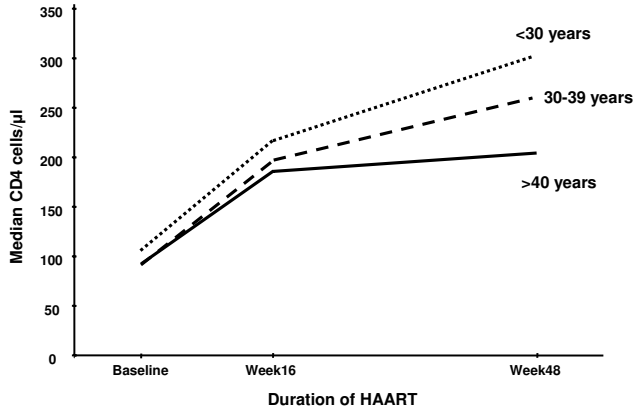
		Phase 1 (0–16 weeks) CD4 slope (n = 596)			Phase 2 (16–48 weeks) CD4 slope (n = 311)[1]		
		Mean	(95% CI)	P value	Mean	(95% CI)	P value
Age (years)	<30	1.0			1.0		
	30–39	-2,27	(-6.71,2.17)	**0.32**	-2.18	(-5.98, 1.63)	**0.26**
	>40	-2.17	(-7.87, 3.54)	**0.46**	-7.31	(-12.11, -2. 50)	**0.003**
Sex	Female	1.0			1.0		
	Male	-3.60	(-8.22, 1.02)	**0.13**	-0.94	(-4.98, 3.10)	**0.65**
Baseline CD4 coimt (cells/μl)	>150	1.0			1.0		
	100–149	3.85	(-1.63, 9.33)	**0,17**	-2.17	(-7.09, 2.76)	**0.39**
	50–99	3.53	(-1.84, 8.91)	**0.20**	0.93	(-4.22, 6.08)	**0.72**
	<50	2.34	(-3.31, 7.98)	**0.42**	8.55	(2.39, 14.71)	**0.007**
WHO clinical stage	1 & 2	1.0			1.0		
	3	1.79	(-3.33, 6.91)	**0.49**	-0.70	(-5.40, 4.00)	**0.77**
	4	0.55	(-5.39, 6.49)	**0.86**	-1.76	(-6.93, 3.41)	**0.50**
Baseline viral load (copies/ml)	<5 $\log_{10}$	1.0			1.0		
	>5 $\log_{10}$	11.14	(7.10, 15.18)	**<0.001**	2.21	(-1.37, 5.80)	**0.23**

[1] The Phase 2 model included as covariates all variables shown, as well the phase 1 CD4 cell slope, the CD4 cell count at 16 weeks and the viral load at 16 weeks (see text).



Baseline CD4 cell count (cells/µl)	Phase 1 (0-16 weeks)	Phase 2 (16-48 weeks)
≥150	21.9 (9.3, 42.9)	5.1 (-3.0, 15.5)
100-149	24.5 (13.4, 44.2)	5.3 (-2.1, 13.5)
50-99	26.0 (11.4, 42.9)	6.8 (-0.2, 14.6)
<50	26.0 (17.8, 40.8)	9.9 (2.3, 21.3)

Figure 2
Graph showing the median CD4 cell counts at baseline and during ART stratified by baseline CD4 cell count. Below the graph, the median (IQR) rates of CD4 cell increase (cells/µl/month) are given for phase 1 (0 – 16 weeks) and phase 2 (16–48 weeks) of immune recovery.



Patient age (years)	Phase 1 (0-16 weeks)	Phase 2 (16-48 weeks)
<30	26.8 (12.7, 43.3)	8.4 (1.1, 20.4)
30-39	24.8 (11.8, 43.0)	6.6 (-1.2, 16.9)
≥40	23.3 (13.1, 38.0)	4.8 (-1.4, 10.6)[1]

Figure 3
Graph showing the median CD4 cell counts at baseline and during ART stratified by age. Below the graph, the median (IQR) rates of CD4 cell increase (cells/µl/month) are given for phase 1 (0 – 16 weeks) and phase 2 (16–48 weeks) of immune recovery.

Among patients with baseline CD4 cell counts of <50, 50–99, 100–149 and 150 cells/µl, the proportions of patients who were immunological non-responders were 5%, 4%, 11% and 19%, respectively. Furthermore, in multivariate analyses, an increment of <50 cells/µl was independently associated with higher baseline CD4 cell count as well as older age, lower baseline viral load, and a viral load >400 copies/ml at any follow-up time-point (Table 3).

Baseline CD4 cell count and failure to attain a CD4 cell count of ≥ 200 cells/µl

We next determined factors associated with failure to attain an absolute CD4 cell count of 200 cells/µl at 48 weeks. Although patients with a baseline CD4 cell count <50 cells/µl had similar rates of phase 1 CD4 cell recovery and greater rates of phase 2 CD4 cell recovery compared to those with higher baseline counts, such patients nevertheless had a reduced likelihood of attaining a CD4 cell count of 200 cells/µl at 48 weeks (Table 3). The proportions of patients with baseline CD4 cell counts of <50, 50–99, 100–149 and 150 cells/µl who failed to attain a CD4 cell count of 200 cells/µl at 48 weeks were 49%, 35%, 18%, and 9%, respectively. Failure to attain 200 cells/µl was also significantly associated with older age, lower baseline plasma viral load, and a viral load >400 copies/ml at any follow-up time-point (Table 3).

Super-responders

Of 311 patients studied out to 48 weeks, 21 (6.8%) achieved an absolute CD4 cell count of >500 cells/µl. These super-responders were principally characterised by age <40 years and by all having follow-up viral loads persistently suppressed <50 copies/ml and a CD4 cell count of 150 cells/µl after 16 weeks of ART. This group of patients had a wide distribution of baseline CD4 cell counts, and included among them were 4 (19%) who had baseline CD4 cell counts of <50 cells/µl. Thus, a low baseline CD4 cell count did not preclude patients from having an excellent immunological response.

Discussion

To our knowledge this is the first analysis to examine the determinants of CD4 cell count recovery among patients receiving ART in resource limited settings. These data indicate that those with baseline CD4 cell counts <50 cells/µl had similar rates of phase 1 CD4 recovery (0–16 weeks) and greater rates of phase 2 recovery (16–48 weeks) compared to rates among those with higher baseline CD4 cell counts. Moreover, those with the lowest baseline counts

Table 3: Results of logistic regression models predicting overall change in blood CD4 cell count during ART. Responses are defined as either (i) the risk of immunological non-response (an increase of <50 cells/μl) or (ii) failure to attain an absolute CD4 cell count of ≥ 200 cells/μl after 48 weeks ART.

		Risk of immumological non-respouse at 48 weeks			Risk of failure to attain CD4 cell count of ≥ 200 cells/μl at 48 weeks		
		OR	**(95% CI)**	**P value**	**OR**	**(95% CI)**	**P value**
Age (years)	<30			1.0	1.0		
	30–39	3.15	(1.09, 9.15)	**0.035**	1.34	(0.66, 2.72)	**0.41**
	>40	3.88	(1.13, 13.39)	**0.032**	3.52	(1.55, 7.99)	**0.003**
Sex	Female	1.0					
	Male	0.42	(0.13, 1.39)	**0.16**	1.73	(0.90, 3.35)	**0.10**
Baseline CD4 count	>150	1.0			1.0		
	100–149	0.63	(0.23, 1.72)	**0.37**	2.49	(0.89, 6.96)	**0.08**
	50–99	0.18	(0.05 0.70)	**0.013**	5.97	(2.29, 15.58)	**<0.001**
	<50	0.18	(0.05, 0.72)	**0.016**	12.11	(4.43, 33.14)	**<0.001**
WHO clinical stage	1 & 2	1.0			1.0		
	3	1.12	(0.37, 3.40)	**0.84**	1.32	(0.54, 3.26)	**0.54**
	4	2.11	(0.63, 7.03)	**0.22**	1.19	(0.45, 3.17)	**0.72**
Baseline viral load	<5 $\log_{10}$	1.0			1.0		
	>5 $\log_{10}$	0.21	(0.07, 0.66)	**0.008**	0.47	(0.26, 0.86)	**0.014**
Follow-up viral load	<400 all visits	1.0			1.0		
	>400 any visit	4.25	(1.30, 13.87)	**0.017**	3.00	(1.14, 7.90)	**0.026**

were least likely to be immunological non-responders to ART. Despite these observations, those with baseline CD4 cell counts <50 cells/μl were nevertheless least likely to attain a CD4 cell count 200 cells/μl at 48 weeks. Taken together, these results suggest that although patients with very low baseline CD4 cell counts retain capacity for similar or slightly greater rates of CD4 cell count recovery compared to those with higher counts, they are nevertheless likely to require a longer period of time to attain a CD4 cell count threshold of 200 cells/μl. Thus, a prolonged period below a 'safe' CD4 cell count threshold rather than a diminished rate of immunological recovery is likely to underlie the high rates of morbidity and mortality observed among those with advanced disease during the early months of ART [4,5,8].

The findings of this analysis are strengthened by the relatively homogeneous study population receiving treatment at a single facility using standardised clinical protocols. Patients were all ART-naïve and received a standard triple-drug regimen with uniform follow-up time points. Quality-assured laboratory assays were all performed in a single nationally accredited laboratory. In contrast, previous studies of the determinants of CD4 cell count recovery have examined heterogeneous study populations in multiple centres and used diverse treatment regimens. Moreover, these studies included many patients with prior ART exposure [20-22] and some included only those who maintained suppression of plasma HIV load [9,23,24]. Our patient population was treated under the government ART roll-out programme and data are therefore likely to be generalisable to other ART programmes in sub-Saharan Africa. Our study is limited to analysis of CD4 cell recovery during the first year of ART and long-term outcomes and their sustainability remain to be determined. Moreover, in the present analysis we have examined recovery of CD4 cell counts but not CD4 cell functional responses.

The immunological response to ART among those with low CD4 cell counts was excellent with the proportion of patients with a CD4 cell count <100 cells/μl decreasing from 51% at baseline to just 4% at 48 weeks. However, our most important finding was that in multivariate analysis baseline CD4 cell counts <50 cells/μl were independently associated with similar rates of phase 1 CD4 cell recovery and greater rates of phase 2 CD4 cell recovery compared to individuals with higher baseline CD4 cell counts. This has not been clearly highlighted in previous publications from Europe and North America although comparison of our data with previous studies is difficult in view of differing cohort compositions. However, this overall observation is consistent with the findings of Bennett *et al.*[21], and Le Moing *et al.* showed a similar but non-significant trend when comparing patients with baseline CD4 cell counts <200 cells/μl with those with higher counts [20]. Kaufmann *et al.* found that CD4 cell increases in the first year of ART were similar comparing those with baseline counts <100 cells/μl with those with counts 100–199 cells/μl [22].

Survival bias could potentially have affected our findings. We have previously shown in this cohort that patients with the lowest baseline CD4 cell counts had a higher risk of death [5] and immunological non-responders or poor

responders may have a greater mortality risk, leading to a survivor effect. However, we were careful to ensure that rates of CD4 cell recovery in the CD4 cell strata did not differ when comparing analyses of the whole cohort with those for whom data points were available at every time-point. Secondly, WHO clinical stage of disease is the strongest predictor of death in this cohort [5] and yet this variable was not associated with CD4 cell responses. Thirdly, the majority of deaths occurred in the first few weeks of ART among those whose disease was simply too far advanced; such deaths probably do not reflect a lack of immunological response to ART. Finally, we have previously reported that over 20% of early deaths in this cohort are due to immune reconstitution disease [5,25]; such deaths typically occur among those with low baseline CD4 cell counts and good CD4 cell recovery. These deaths would tend to cause exactly the opposite bias. Thus, although an important consideration, we do not think that survival bias had an overall dominant effect.

We have previously shown that low baseline CD4 count at entry to an ART programme was associated with increased risks of tuberculosis and of mortality during the first year of ART [5,8]. Results from the present study suggest that these increased risks are likely to reflect an increased period of time required for such patients to achieve a 'safe' level of immune function rather than reflecting impaired rates of immune recovery and this is consistent with the findings of a study from Spain with longer term follow-up [11]. More recently we have found that risk of mortality and risk of incident tuberculosis is strongly associated with the current CD4 cell count during ART rather than the baseline count (unpublished data). Thus, if patients with profound baseline CD4 lymphocytopenia survive the initial few months of treatment and achieve full viral load suppression, then high rates of immune recovery are likely to result in a better prognosis that might have been anticipated.

Rates of phase 1 and phase 2 CD4 cell increase were similar in magnitude to those previously reported from high-income countries [9,20]. The rapid phase 1 CD4 cell recovery was strongly associated with baseline viral load as described previously [20,21]; patients with viral loads $>10^5$ $\log_{10}$ copies/ml had 11-fold greater CD4 count increases than those with lower viral loads. Immune dysregulation and immune cell activation promote sequestration of $CD4^+CD45Ro^+$ memory T cells in lymphoid tissue; suppression of viral replication then triggers rapid redistribution of this cell pool and a reduction in apoptotic cell death during the initial weeks of ART [26,27]. A positive correlation between the plasma viral load and the degree of cell sequestration may provide a possible mechanism underlying the observation that those with the highest viral loads have the greatest initial CD4 cell increment. The fact that patients with profound CD4 lymphocytopenia have good immunological recovery during ART is likely to be important in the pathogenesis of immune reconstitution disease associated with *Mycobacterium tuberculosis*[28], *Cryptococcus neoformans*[25] that is frequently observed in this patient population.

Greater phase 2 CD4 cell recovery was strongly associated with age as reported elsewhere [9,24]. Sustained suppression of viral replication is also critical to second phase recovery [20,21] and we confirmed that viral load at 16 weeks was a strong independent predictor of subsequent immunological recovery. Ten per cent of patients were immunological non-responders (CD4 cell increment <50 cells/μl at 48 weeks) and 7% of patients had discordant responses, having immunological non-response despite a fully suppressed viral load. These rates are much lower than those reported in previous series from industrialised countries [14,29], possibly because our study only included antiretroviral-naïve patients, rates of HIV primary drug resistance in this setting are likely to be low, and because rates of treatment adherence in our cohort are very high. However, those with CD4 cell counts <50 cells/μl were least likely to be immunological non-responders. Moreover, 19% of 'super-responders' had baseline CD4 cell counts <50 cells/μl, indicating that a very low baseline CD4 cell count does not preclude an excellent CD4 cell count response to ART.

Conclusion

Patients with the lowest CD4 counts in this setting do not have diminished capacity for immune recovery. Although patients with low baseline CD4 counts have increased risk of acute morbidity and mortality, if such patients survive the initial months of ART and fully suppress the viral load, their chances of immunological recovery are good during the first year. Future studies are required to determine the long-term prospects for immune recovery among patients treated in ART programmes in sub-Saharan Africa.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

SDL and RW initiated and designed the study. LM did the statistical analyses. LGB and RW established the study cohort and data collection systems. SDL wrote the manuscript, which RW, LM and LGB all helped to revise.

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Major Clinical Outcomes in Antiretroviral Therapy (ART)–Naive Participants and in Those Not Receiving ART at Baseline in the SMART Study

The Strategies for Management of Antiretroviral Therapy (SMART) Study Group[a]

(See the editorial commentary by Hughes and Ribaudo, on pages 1084–6.)

Background. The SMART study randomized 5472 human immunodeficiency virus (HIV)–infected patients with $CD4^+$ cell counts >350 cells/μL to intermittent antiretroviral therapy (ART; the drug conservation [DC] group) versus continuous ART (the viral supression [VS] group). In the DC group, participants started ART when the $CD4^+$ cell count was <250 cells/μL. Clinical outcomes in participants not receiving ART at entry inform the early use of ART.

Methods. Patients who were either ART naive ($n = 249$) or who had not been receiving ART for ⩾6 months ($n = 228$) were analyzed. The following clinical outcomes were assessed: (i) opportunistic disease (OD) or death from any cause (OD/death); (ii) OD (fatal or nonfatal); (iii) serious non-AIDS events (cardiovascular, renal, and hepatic disease plus non–AIDS-defining cancers) and non-OD deaths; and (iv) the composite of outcomes (ii) and (iii).

Results. A total of 477 participants (228 in the DC group and 249 in the VS group) were followed (mean, 18 months). For outcome (iv), 21 and 6 events occurred in the DC (7 in ART-naive participants and 14 in those who had not received ART for ⩾6 months) and VS (2 in ART-naive participants and 4 in those who had not received ART for ⩾6 months) groups, respectively. Hazard ratios for DC vs. VS by outcome category were as follows: outcome (i), 3.47 (95% confidence interval [CI], 1.26–9.56; $P = .02$); outcome (ii), 3.26 (95% CI, 1.04–10.25; $P = .04$); outcome (iii), 7.02 (95% CI, 1.57–31.38; $P = .01$); and outcome (iv), 4.19 (95% CI, 1.69–10.39; $P = .002$).

Conclusions. Initiation of ART at $CD4^+$ cell counts >350 cells/μL compared with <250 cells/μL may reduce both OD and serious non-AIDS events. These findings require validation in a large, randomized clinical trial.

Trial registration. ClinicalTrials.gov identifier: NCT00027352.

The most recent randomized clinical trial that addressed the question of when to start antiretroviral therapy (ART) for the treatment of HIV disease was the Concorde study [1]. Since then, despite enormous advances afforded through the development of antiretroviral drugs and the use of combination regimens, there has been no randomized study to address this critically important issue. The absence of randomized data in both the developed and developing world has required antiretroviral treatment guidelines to rely on the interpretation of nonrandomized studies and expert opinions [2, 3]. Current recommendations for the initiation of ART are conservative because of the uncertainty about the risk-benefit ratio associated with earlier ART use at higher $CD4^+$ cell counts. These include toxicities, cost-effectiveness, quality of life issues, adherence, and drug resistance. At present, the initiation of ART is recommended for asymptomatic patients with $CD4^+$ cell counts between 201 and 350 cells/μL or, in developing/resource-poor countries, when the $CD4^+$ cell count has declined to ⩽200 cells/μL.

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[a] Study group members (including the members of the Writing Group, who authored this article), conflicts of interest, and the role of the funding source are listed after the text.

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Data from cohort studies indicate that the risk of AIDS decreases with increasing $CD4^+$ cell counts but nevertheless persists at $CD4^+$ cell counts $\geq$500 cells/μL [4]. Furthermore, at any given $CD4^+$ cell count, the risk of AIDS appears to be lower in patients treated with ART than in those who are not [5], and the risk of AIDS or death declines with the start of therapy, even among those with high $CD4^+$ cell counts at initiation, compared with that in patients not receiving ART [6, 7]. Importantly, cohort studies also indicate that risks of serious non–AIDS-related diseases, including cardiovascular, renal, and hepatic conditions and non-AIDS malignancies, are less common at higher $CD4^+$ cell counts [8–13]. Data from the SMART study are consistent with these observations insofar as the rates of AIDS and serious non-AIDS events were significantly higher in participants randomized to the intermittent ART group than in those randomized to the continuous ART group [14].

Here, we report the major clinical outcomes for a subgroup of participants enrolled in SMART who were either ART naive or who had not been receiving ART for $\geq$6 months before randomization. This subgroup provides a randomized comparison between early ART at a $CD4^+$ cell count of $>$350 cells/μL versus deferred ART until the $CD4^+$ count declines to $<$250 cells/μL. Subgroup analyses for major clinical outcome according to ART history were proposed a priori. However, the analyses presented here are post hoc. Thus, we regard our results as hypothesis generating.

METHODS

Participants. Participants were included if they were randomized in the SMART study and were either ART naive or satisfied the following eligibility criteria for having been previously treated but not receiving ART for a minimum of 6 months before randomization (hereafter, "not receiving ART"): (1) were not receiving ART at the time of randomization in SMART; (2) had at least 1 plasma HIV RNA assessment during the 6 months before randomization; and (3) all plasma HIV RNA levels in the 6 months before randomization were $>$10,000 copies/mL. We excluded patients who had not been receiving ART for $<$6 months before randomization to avoid the possible short-term effects of ceasing ART (e.g., the steeper decline in $CD4^+$ cell count). The principal eligibility criterion for the SMART study was a $CD4^+$ cell count of $>$350 cells/μL. Other eligibility criteria for SMART are described elsewhere [14].

Randomization. Participants were randomly allocated in equal proportions to 1 of 2 treatment strategies: continuous ART (the viral supression [VS] group) and intermittent ART (the drug conservation [DC] group). For the present analyses, this randomization corresponded to the immediate (re)initiation of ART after randomization at $CD4^+$ cell counts $>$350 cells/μL (the VS group) versus the deferred (re)initiation of ART when either the $CD4^+$ cell count declined to $<$250 cells/μL, the $CD4^+$ cell percentage declined to $<$15%, or symptoms of HIV disease developed (the DC group).

Assessments. Before randomization, participants' ART and medical history were obtained along with nadir and baseline $CD4^+$ cell counts and plasma HIV RNA levels. Routine visits occurred at 1 and 2 months, every 2 months thereafter for the first year, and every 4 months in subsequent years. Visits included clinical assessments, and samples were obtained for measurement of $CD4^+$ cell count and plasma HIV RNA level. At baseline and annually, a 12-lead electrocardiogram was obtained, and data were electronically transmitted to a central reading facility for assessment of silent myocardial infarction [15].

End-point definitions. Definitions for the primary end point of opportunistic disease (OD) or death from any cause (OD/death) and the major secondary composite end point of cardiovascular-, renal-, and hepatic-disease events are given in the online-only appendix of the primary-results article [14]. In the present report, 4 major clinical outcomes are considered: (i) the SMART primary end point (OD/death); (ii) OD (fatal or nonfatal); (iii) serious non-AIDS events (cardiovascular, renal, and hepatic disease plus non–AIDS-defining cancers [excluding nonmelanoma skin cancers] and deaths from non-OD causes); and (iv) the composite of outcomes (ii) and (iii). In the end-point categories (ii) and (iii), deaths due to OD and all causes except OD are counted. Thus, the composite of (ii) and (iii) can be viewed as major morbidity (OD and non-OD) or all-cause mortality. As previously reported, an end-point review committee, blinded to treatment group, reviewed all major nonfatal clinical events [14]. The end-point review committee also classified the underlying cause of death using the system of the Coding of Death in HIV Project [16]. Grade 4 adverse events were defined as potentially life-threatening symptomatic events according to the Division of AIDS common toxicity table.

Statistical analysis. All analyses were intention to treat. Data through 11 January 2006 were reported. On that date, following the recommendations of an independent data and safety monitoring board, all participants in the DC group were advised to (re)initiate ART. Thus, time-to-event analyses are censored at the earliest of the date of death, the lost to follow-up date, or 11 January 2006.

The time from randomization to first event was compared between the DC and VS groups by use of Kaplan-Meier plots for each event category. For patients reporting multiple events in a single outcome category, only the first event was included. For patients reporting multiple events in different outcome categories, each first event in each category was included. The hazard ratio for DC vs. VS—hereafter, HR(DC/VS)—was estimated by fitting a Cox model with a single binary indicator for treatment group overall and separately in ART-naive and ART-experienced participants. Differences between these 2 groups were assessed by including an interaction term in the Cox model.

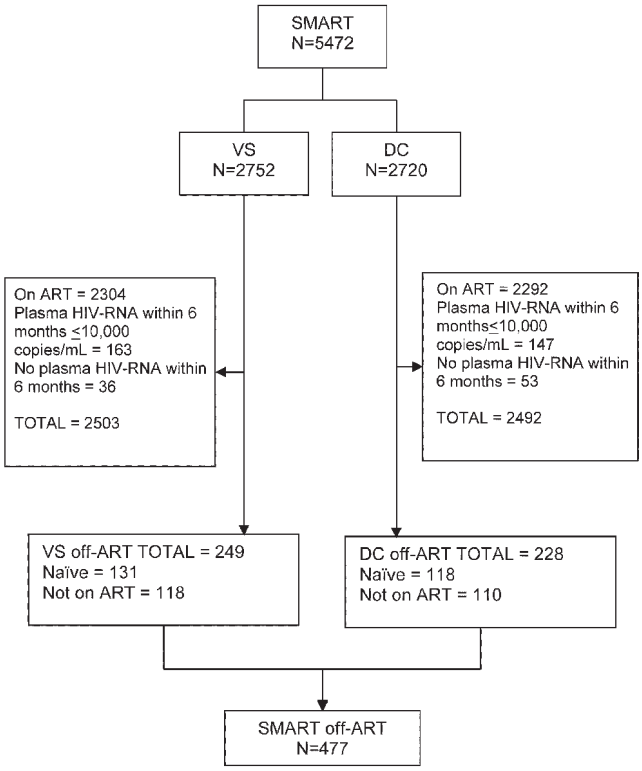

Figure 1. Selection of the analyzed cohort (antiretroviral therapy [ART] naive or not receiving ART for ⩾6 months) from the overall population of SMART participants. DC, drug conservation; VS, viral supression.

Randomization protects these comparisons between treatment groups from confounding.

Person-years at specific $CD4^+$ cell counts were counted according to the most recently measured or time-updated value (latest $CD4^+$ cell count), and rates per 100 person-years for the composite outcome were determined for DC and VS participants. Randomization does not protect these comparisons between treatment groups from confounding.

Throughout, *P* values refer to 2-sided tests. Because the subgroup and the serious non-AIDS outcomes considered in this report were not prespecified, we recommend cautious interpretation of *P* values.

RESULTS

Patient population. A total of 477 participants (8.7%) in the SMART study were either ART naive ($n = 249$) or had not been receiving ART for ⩾6 months before randomization ($n = 228$) and are included in these analyses. The relationship of these participants to the total SMART cohort is summarized in figure 1.

Table 1 shows the baseline characteristics of the 228 and 249 participants who were respectively randomized into the DC and VS groups. Treatment groups were well balanced for all baseline characteristics. One-hundred eighteen participants (52%) and 131 participants (53%) were ART naive in the DC and VS groups, respectively. The median baseline $CD4^+$ cell count and plasma HIV RNA level for the cohort were 447 cells/μL and 4.5 $\log_{10}$ copies/mL. The median nadir $CD4^+$ cell count was 361 cells/μL.

The median duration of ART exposure in previously treated participants was 4 years. Approximately 75% had used an HIV protease inhibitor (PI), for a median duration of 2.5 years. Approximately 50% had used a nonnucleoside reverse-transcriptase inhibitor (NNRTI), for a median duration of 2.5 years. All previously treated participants had used a nucleoside reverse-transcriptase inhibitor (NRTI).

In the VS group, (re)initiation of ART comprising NRTIs in combination with NNRTIs, NRTIs in combination with a PI, and NRTIs alone was reported for 134 (56%), 73 (31%), and 31 (13%) participants, respectively.

There were modest differences in baseline characteristics between participants who were ART naive and those who were experienced. ART-naive participants were on average 3 years younger, less likely to be current smokers, less likely to be taking blood-pressure-lowering or lipid-lowering therapy, and had slightly lower serum triglyceride levels. They were also less likely to be coinfected with either hepatitis B or C virus and less likely to have had a prior AIDS-defining illness.

The mean period of follow-up was 18 months (median, 15 months). Eleven (4 DC and 7 VS) participants were lost to follow-up.

Exposure to ART. DC and VS participants received ART for 18% and 89% of the follow-up time, respectively. In the DC group, 68 participants (30%) (re)initiated ART, 25 of whom were ART naive (21% of the ART-naive participants) and 43 of whom were ART experienced (39% of the ART-experienced participants). Reasons for (re)initiation of ART included $CD4^+$ cell count decline to <250 cells/μL (38 participants [56% of those who (re)initiated ART]); $CD4^+$ cell percentage decline to $<15\%$ (28 participants [41%]); symptoms of HIV disease or development of OD (14 participants [21%]), with 4 participants developing an OD event; and nonprotocol reasons (14 participants [21%]). Of these 68 participants, 30 (44%) initiated ART at a $CD4^+$ cell count ⩾250 cells/μL. Kaplan-Meier estimates for DC participants (re)initiating ART were 11%, 25%, 39%, 45%, 47%, and 55% at 6, 12, 18, 24, 30, and 36 months, respectively. In the DC group, participants (re)initiated ART at a median $CD4^+$ cell count of 236 cells/μL (interquartile range [IQR], 183–310 cells/μL); those who were ART naive initiated ART at median of 245 cells/μL (IQR, 192–338 cells/μL), versus 225 cells/μL (IQR, 180–307 cells/μL) among ART-experienced participants. In comparison, 238 participants (96%) in the VS group (re)initiated ART at study entry, at a $CD4^+$ cell count ~200 cells/μL higher; 189 (79%) had a $CD4^+$ cell count between 350 and 549 cells/μL, and 49 (21%) had a $CD4^+$ cell count ⩾550 cells/μL.

In the DC group, 68 (30%) of 228 participants started ART, and 27 (12%) subsequently interrupted ART. The majority of

Table 1. Overall baseline characteristics and those for patients who were antiretroviral therapy (ART) naive or who had not received ART for ⩾6 months (no ART) before randomization in the SMART study.

Characteristic	DC group (n = 228)			VS group (n = 249)			Total (n = 477)		
	ART naive (n = 118)	No ART (n = 110)	Overall	ART naive (n = 131)	No ART (n = 118)	Overall	ART naive (n = 249)	No ART (n = 228)	Overall (IQR)
Age, median, years	40	41	41	39	43	41	39	42	41 (35–47)
Female	20.3	22.7	21.5	27.5	32.2	29.7	24.1	27.6	25.8
Race/ethnicity									
Black	32.2	39.1	35.5	35.9	39.0	37.3	34.1	39.0	36.4
White	54.2	48.2	51.3	45.8	48.3	47.0	49.8	48.3	49.1
Other	13.6	12.7	13.2	18.3	12.7	15.7	16.1	12.7	14.5
Mode of infection									
Same sex contact	55.1	53.6	54.4	48.1	53.4	50.6	51.4	53.5	52.4
Opposite sex contact	44.9	45.5	45.2	52.7	42.4	47.8	49.0	43.9	46.5
Injection drug use	6.8	18.2	12.3	7.6	11.9	9.6	7.2	14.9	10.9
Other or unknown	6.8	7.3	7.0	12.2	11.0	11.6	9.6	9.2	9.4
Body mass									
Weight, median, kg	76.7	73.9	75.3	76.7	78.0	77.1	76.7	75.5	76.2 (67.5–87.6)
BMI, median	25.1	24.8	24.9	25.6	25.8	25.8	25.2	25.4	25.3 (22.7–28.8)
$CD4^+$ cell count, median, cells/μL									
Nadir	371	339	358	378	347	365	376	341	361 (300–422)
Baseline	441	458	452	432	447	435	437	452	447 (385–536)
Baseline plasma HIV RNA level, median, $\log_{10}$ copies/mL	4.6	4.7	4.6	4.3	4.6	4.5	4.4	4.6	4.5 (4.1–4.9)
Prior AIDS	5.9	14.5	10.1	7.6	16.1	11.6	6.8	15.4	10.9
Current smoker	44.9	51.8	48.2	42.7	50.0	46.2	43.8	50.9	47.2
Diabetes	5.9	6.4	6.1	3.8	5.9	4.8	4.8	6.1	5.5
Prior cardiovascular disease	2.5	0.9	1.8	1.5	4.2	2.8	2.0	2.6	2.3
Blood-pressure-lowering drugs	7.6	20.9	14.0	16.8	17.8	17.3	12.4	19.3	15.7
Lipid-lowering drugs	5.9	6.4	6.1	3.8	7.6	5.6	4.8	7.0	5.9
Lipid levels, median, mg/dL									
Total cholesterol	163	163	163	164	171	166	164	164	164 (142–193)
HDL cholesterol	35	36	36	38	33	36	36	35	36 (29–45)
LDL cholesterol	98	94	96	100	99	100	99	96	97 (78–123)
Triglyceride	120	126	124	121	144	132	121	135	128 (87–192)
ART history									
ART naive	100.0	0.0	51.8	100.0	0.0	52.6	100.0	0.0	52.2
PI experienced	0.0	75.5	36.4	0.0	69.5	32.9	0.0	72.4	34.6
NNRTI experienced	0.0	47.3	22.8	0.0	55.9	26.5	0.0	51.8	24.7
Coinfection									
Hepatitis B virus	0.9	4.6	2.7	1.6	5.2	3.3	1.2	4.9	3.0
Hepatitis C virus	8.5	24.5	16.2	13.0	16.9	14.9	10.8	20.6	15.5

NOTE. Data are percentage of participants, unless otherwise indicated. BMI, body mass index; DC, drug conservation; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; VS, viral supression.

follow-up time was accrued before the first (re)initiation of ART: 236 person-years (79%) accrued from study entry to first (re)initiation of ART, 39 person-years (13%) from (re)initiation of ART to discontinuation, and 25 person-years (8%) after discontinuation of ART. During this 8% of follow-up time, 3 participants in the DC group experienced an OD event, a serious non-AIDS event, or died.

$CD4^+$ cell count and plasma HIV RNA outcomes. Reflecting the differing ART exposures, there were substantial differences in $CD4^+$ cell counts and plasma HIV RNA levels over time between the DC and VS groups. Figure 2 shows time curves for $CD4^+$ cell count and plasma HIV RNA level for each treatment group. The $CD4^+$ cell count was, on average, 148 cells/μL lower in DC than VS participants. The proportions of participants with plasma HIV

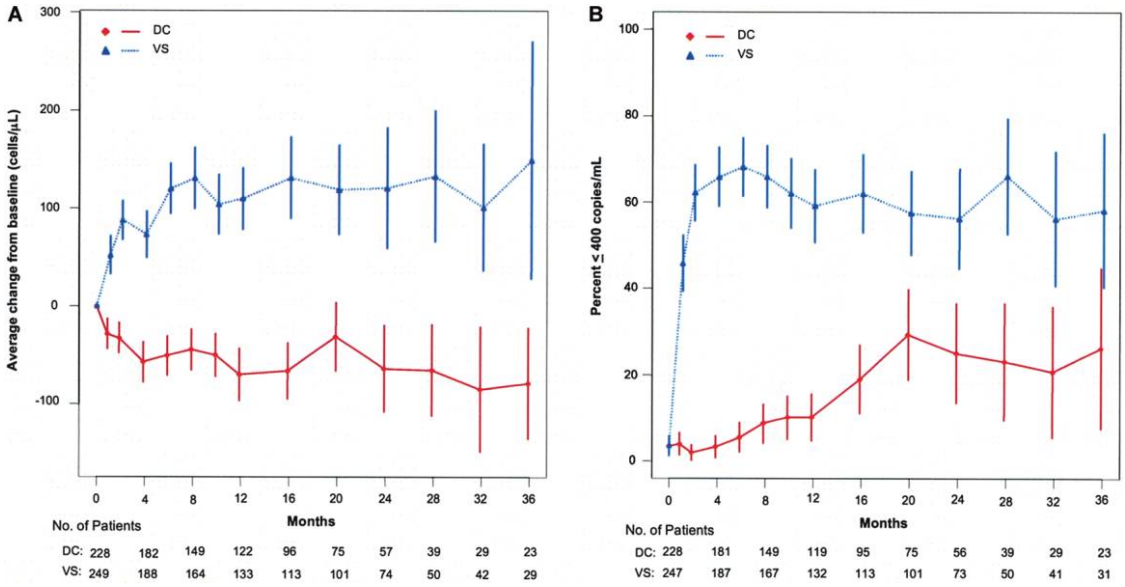

Figure 2. Mean change from baseline CD4⁺ cell count *(A)* and percentage of patients with plasma HIV RNA levels ⩽400 copies/mL *(B)* during follow-up. The solid red lines indicate participants in the drug conservation (DC) group, and the dashed blue lines indicate participants in the viral supression (VS) group. Vertical bars represent ± 2 standard errors.

RNA levels ⩽400 copies/mL at 12 and 24 months were 10% and 25% in the DC group and 59% and 56% in the VS group.

Clinical outcomes. Fifteen participants in the DC group and 5 in the VS group experienced OD/death (HR, 3.5 [95% confidence interval {CI}, 1.3–9.6) (figures 3 and 4*A*). The corresponding HR(DC/VS) for OD and for serious non-AIDS events was 3.3 (95% CI, 1.0–10.3) and 7.0 (95% CI, 1.6–31.4), respectively (figures 3, 4*B*, and 4*C*). There was relatively little overlap in

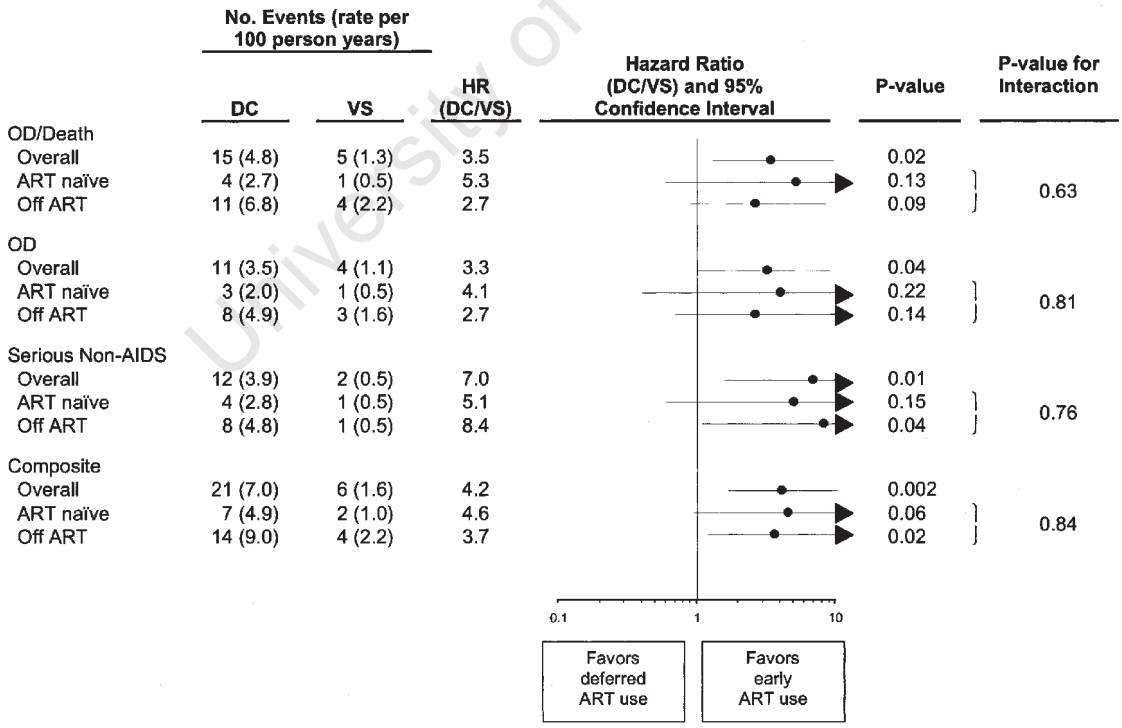

Figure 3. Hazard ratios (HRs) for clinical outcomes among the overall cohort and among those who were either antiretroviral therapy (ART) naive or not receiving ART at baseline. Horizontal lines represent the 95% confidence interval for each HR. The *P* value for interaction compares event rates within each category for ART-naive and ART-experienced participants. DC, drug conservation; VS, viral supression.

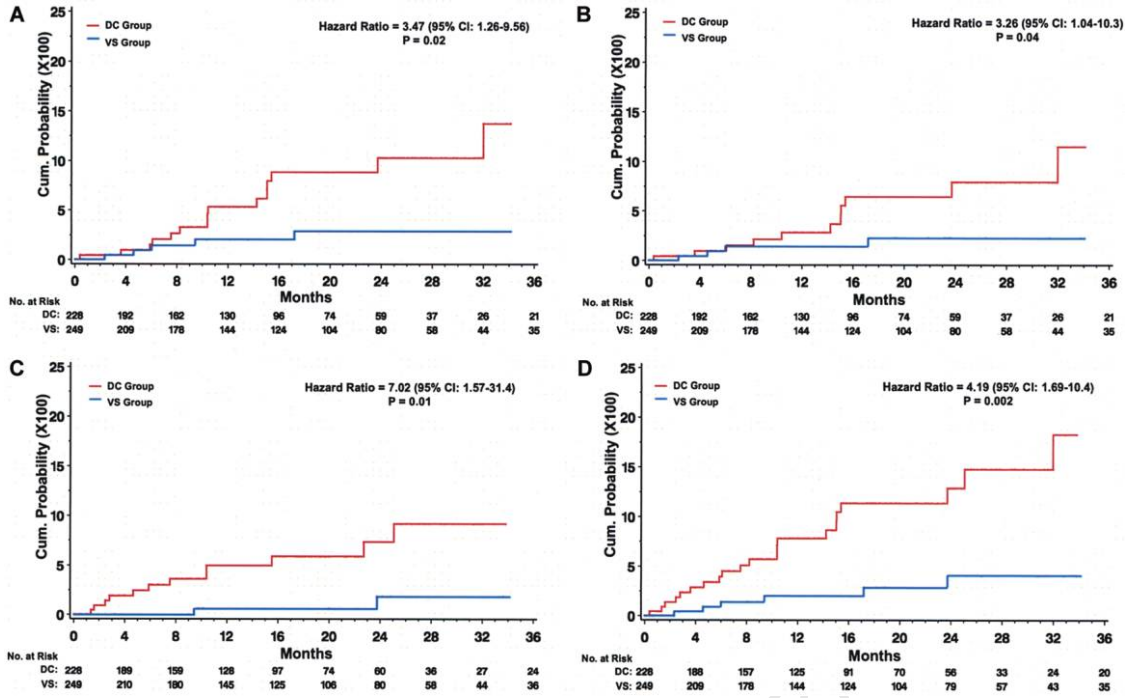

Figure 4. Kaplan-Meier time curves for cumulative probability of opportunistic disease (OD) and death *(A),* OD alone *(B),* serious non-AIDS events and non-OD death *(C),* and the composite of OD and serious non-AIDS events, which includes all-cause death *(D).* The solid red lines indicate events in participants in the drug conservation (DC) group, and the dashed blue lines indicate events in participants in the viral supression (VS) group.

OD and serious non-AIDS events (table 2); 21 participants in the DC group and 6 in the VS group experienced at least 1 of these (the composite end point) (figures 3 and 4*D*). The HR for the composite outcome was 4.2 (95% CI, 1.7–10.4). The excess risk (DC minus VS) was 5.4 events/100 person-years (95% CI, 3.4–7.4). The cumulative percentage developing the composite end point by 12, 24, and 36 months were 8%, 13%, and 18% for DC participants and 2%, 4%, and 4% for VS participants (figure 4*D*).

For the composite end point, HRs were 4.6 (95% CI, 1.0–22.2) for the ART-naive participants and 3.7 (95% CI, 1.2–11.2) for the ART-experienced participants ($P = .84$ for difference in these HRs). In participants excluded from this cohort (see figure 1), HRs for the composite end point were 1.3 (95% CI, 0.6–3.2) for experienced participants not receiving ART at entry (200 DC and 199 VS participants) and 2.0 (95% CI, 1.5–2.6) for participants receiving ART at entry (2292 DC and 2304 VS participants) ($P = .27$ for the difference in HRs between these 4 subgroups). In the entire SMART study, there were 270 participants with a composite end point (180 DC and 90 VS participants), with a corresponding HR of 2.1 (95% CI, 1.6–2.7).

Table 3 summarizes the composite outcome by treatment group and proximal $CD4^+$ cell count. Event rates were higher during the time when participants experienced lower $CD4^+$ cell counts. Per design, participants in the DC group, relative to those in the VS group, spent substantially longer periods of time with $CD4^+$ cell counts in the <350 cells/μL stratum (28% vs. 11% of the time) and substantially less time with $CD4^+$ cell counts in the ≥ 500 cells/μL stratum (32% vs. 61% of the time). The majority of events in the DC group (57%) occurred at proximal $CD4^+$ cell counts ≥ 350 cells/μL. Nine (75%) of 12 events in the DC group (1 OD and 8 serious non-AIDS events) that occurred at $CD4^+$ cell counts ≥ 350 cells/μL happened before the (re)initiation of ART. Table 3 also summarizes the composite outcome in the subgroup of participants without prior AIDS and without a prior non–AIDS-defining illness, for which the HR(DC/VS) was 6.0 (95% CI, 1.7–20.8) and 7.5 (95% CI, 2.2–25.4), respectively. Although event rates were similar or reduced compared with the overall cohort, the HR(DC/VS) did not decrease after excluding participants with these prior events.

We investigated whether differences in nadir $CD4^+$ cell counts might have affected the distribution of clinical events. The data in table 1 indicate that median nadir $CD4^+$ cell counts were not substantially different for the ART-naive or previously treated participants between treatment groups. The differences between ART-experienced and ART-naive participants with respect to median baseline $CD4^+$ cell count (437 and 376 cells/μL, respectively) and median nadir $CD4^+$ cell count (452 and 341 cells/μL, respectively) participants were not appreciable. The HR(DC/VS) for our primary outcome was unaffected in analyses that did or did not adjust for nadir $CD4^+$ cell count (HR of 3.7 in both analyses).

Grade 4 events were experienced by 25 DC participants and 18 VS participants. The HR(DC/VS) was 1.6 (95% CI, 0.9–3.0).

Table 2. Summary of clinical-event diagnoses in the SMART study, by event category and randomized treatment group.

Category, treatment group, patient	Event diagnosis	ART naive	Prior AIDS	Baseline $CD4^+$ cell count, cells/μL	Most recent prior $CD4^+$ cell count, cells/μL	Most recent prior Plasma HIV RNA level, copies/mL
OD						
DC group						
1	**Herpes zoster**	Yes	No	479	498	400
2	**Herpes zoster**	Yes	Yes	600	456	41,400
3	Oesophageal candidiasis	Yes	No	696	696	244,000
4	Oesophageal candidiasis	No	Yes	448	404	5210
5	Tuberculosis	No	No	393	221	534,279
6	Oesophageal candidiasis	No	Yes	387	342	41,846
7	**Oesophageal candidiasis**	No	No	408	254	34,700
7	*Mycobacterium avium* complex	No	No	408	338	130,000
7	**Herpes simplex**	No	No	408	48	17,600
7	Death due to OD	No	No	408	48	17,600
8	Kaposi sarcoma	No	No	368	288	750,000
9	**Tuberculosis**	No	No	458	337	720,000
10	Lymphoma	No	No	801	362	668,000
10	**Death due to NHL**	No	No	801	307	50
11	*Pneumocystis jirovecii* pneumonia	No	No	400	310	388,394
VS group						
12	Tuberculosis	Yes	Yes	399	80	515,705
13	Death due to OD (bacterial pneumonia)	No	No	588	426	81,375
14	Oesophageal candidiasis	No	Yes	518	296	30,400
14	Bacterial pneumonia	No	Yes	518	533	90,700
15	**Kaposi sarcoma**	No	Yes	586	428	2228
Serious non-AIDS events						
DC group						
16	Hepatic cirrhosis	Yes	Yes	627	463	8800
17	End-stage renal disease	Yes	No	379	233	109,000
18	Death due to CVD	Yes	No	492	415	154,000
19	**Myocardial infarction**	Yes	No	390	330	4370
19	**Coronary artery disease surgery**	Yes	No	390	330	4370
20	**Death due to CVD**	No	Yes	376	248	265,100
21	Silent myocardial infarction	No	No	1271	1132	1140
5	Myocardial infarction	No	No	393	324	124,390
5	Silent myocardial infarction	No	No	393	212	401,672
5	**Coronary artery disease surgery**	No	No	393	212	401,672
6	Death due to digestive system disease	No	Yes	387	620	16,271
22	Accidental death	No	No	1224	488	10,037
23	**Death due to renal disease**	No	No	483	615	75
24	Hepatic cirrhosis	No	Yes	459	467	25,176
25	Non-AIDS cancer	No	No	610	407	134,000
25	Myocardial infarction	No	No	610	377	240,000
VS group						
26	**Hepatic cirrhosis**	Yes	No	701	423	388
27	Death due to non-AIDS cancer	No	No	577	251	26,542

NOTE. Seven patients had multiple events. Statistical analyses included only the first occurrence of the given end point. Clinical events shown in boldface are those diagnosed in patients known to be receiving antiretroviral therapy (ART). CVD, cardiovascular disease; DC, drug conservation; NHL, non-Hodgkin lymphoma; OD, opportunistic disease; VS, viral supression.

Table 3. Clinical events (composite end point), by treatment group and proximal CD4+ cell count.

Patient category, proximal CD4+ cell count	DC group			VS group				
	Person-years[a] (%)	Events[b]	Rate[c]	Person-years[a] (%)	Events[b]	Rate[c]	HR(DC/VS)	95% CI
All participants								
<250 cells/μL	19 (6.4)	3 (2)	16.0	10 (2.6)	1	10.5		
250–349 cells/μL	65 (21.7)	6 (3)	9.3	30 (7.9)	2	6.7		
350–499 cells/μL	118 (39.5)	9 (7)	7.6	108 (28.8)	3	2.8		
⩾500 cells/μL	98 (32.4)	3 (2)	3.1	230 (60.7)	0	0.0		
Overall	300 (100)	21 (14)	7.0	378 (100)	6	1.6	4.2	1.7–10.4
Those without prior AIDS								
<250 cells/μL	16 (5.9)	2 (2)	12.8	8 (2.4)	0	0.0		
250–349 cells/μL	58 (21.6)	5 (2)	8.6	26 (7.7)	1	3.9		
350–499 cells/μL	107 (39.8)	5 (4)	4.7	95 (28.1)	2	2.1		
⩾500 cells/μL	88 (32.7)	3 (2)	3.4	209 (61.8)	0	0.0		
Overall	269 (100)	15 (10)	5.6	338 (100)	3	0.9	6.0	1.7–20.8
Those without a prior non–AIDS-defining illness								
<250 cells/μL	17 (5.9)	3 (2)	18.2	9 (2.5)	1	10.9		
250–349 cells/μL	63 (22.0)	5 (2)	8.0	28 (7.9)	1	3.6		
350–499 cells/μL	116 (40.4)	8 (6)	6.9	103 (29.1)	1	1.0		
⩾500 cells/μL	92 (31.7)	3 (2)	3.3	214 (60.5)	0	0.0		
Overall	288 (100)	19 (12)	6.6	354 (100)	3	0.8	7.5	2.2–25.4

NOTE. CI, confidence interval; DC, drug conservation; HR(DC/VS), hazard ratio for DC group vs. VS group; VS, viral supression.

[a] Time spent in the CD4+ cell count category (censored at event).

[b] First events only. Values in parentheses indicate the no. of events that occurred before the (re)initiation of antiretroviral therapy.

[c] Per 100 person-years.

DISCUSSION

The randomization in the SMART study for this patient subset directly compared the early use of ART (at >350 CD4+ cells/μL) versus the deferred use of ART (at <250 CD4+ cells/μL). To our knowledge, the data presented here represent the first substantive evidence from a randomized trial to inform this critical issue in HIV medicine since 1994 [1]. During follow-up, there was substantially lower exposure to ART in the deferred treatment group (DC). The deferred use of ART was consistent with the protocol in the majority of participants; although the DC strategy included discontinuation of ART when the CD4+ cell count increased to >350 cells/μL, 92% of accrued follow-up was in participants who were either not receiving ART or taking ART for the first time after randomization. In the early ART group (VS), most participants (re)initiated ART with CD4+ cell counts between 350 and 549 cells/μL. We combined data from participants who were ART naive and those who had not been receiving ART for at least 6 months before randomization. Had we been more conservative and included only participants who had not been receiving ART for >12 months before randomization, we would have reduced the overall number of composite events from 27 (21 in DC and 6 in VS participants) to 23 (17 in DC and 6 in VS participants). The calculated HR(DC/VS) would still have indicated a significant benefit for the VS strategy (HR, 3.1 [95% CI, 1.2– 7.9]).

The results suggest that morbidity and mortality associated with HIV is probably higher than previously thought for patients with earlier stages of HIV disease. In DC participants, the rates of OD and serious non-AIDS events were similar. The risk of a composite outcome was >4-fold higher in DC participants than in VS participants. The data indicate that the risk of such events is reduced by early versus deferred use of ART (the absolute risk of an OD or serious non-AIDS event in DC versus VS participants was 7.0 vs. 1.6/100 person-years). This difference in absolute risk, if confirmed in a larger trial, is important. Treatment guidelines for the initiation of ART are conservative because the risk of AIDS (OD in our categories) at high CD4+ cell counts is relatively low and because ART-related toxicities (and other factors) have been assumed to outweigh any likely ART-related benefits. Our data suggest that early ART use may result in substantial reductions in serious non-AIDS events. Relative to the DC group, 289 additional person-years of ART prevented 20 serious clinical events in the VS group. Hence, prevention of 1 event requires 14 extra person-years of ART. When assessed for AIDS events alone, prevention of 1 event requires 42 extra person-years of ART.

The occurrence of AIDS at higher than expected CD4+ cell counts has been described previously [7, 17]. There are fewer

data linking $CD4^+$ cell counts and the risk of non–AIDS-related morbidity and mortality. Some non-AIDS conditions appear to be linked to progressive immunodeficiency [8, 9, 18]. In the SMART study, lower $CD4^+$ cell counts and higher plasma HIV-RNA levels explain much of the excess risk in DC compared with VS participants for non-OD death as well as OD [18]. The data presented here for SMART participants not receiving ART are consistent with these observations.

Mechanistically, it appears to be unlikely that a common pathway for all non-AIDS events exists. Furthermore, the risk and/or the benefit of ART for reducing non-AIDS diseases is not clear. For example, PI therapy has been associated with an increased risk of myocardial infarction [19, 20]. On the other hand, continuous ART in SMART was associated with better cardiovascular outcomes than ART interruption [14]. It is possible that untreated HIV disease supports a range of inflammatory responses that, particularly over long periods, set the scene for atherosclerosis in individuals with additional risk factors for poor cardiovascular outcomes. The increased risk of cardiovascular disease in patients with systemic lupus erythematosus is similar in this regard [21, 22]. In addition, in SMART, changes in lipids appear to explain some of the increased risk of cardiovascular disease in the DC compared with the VS group [23]. The pathogenesis of hepatic and renal disease along with non-AIDS cancer is less clear. These equivocal and perhaps controversial issues further highlight the need for a careful evaluation of risks and benefits of early treatment with ART [24]. Furthermore, they reinforce the importance of studies with systematically collected clinical end points matched with appropriate biological samples as a key feature of trial design for HIV infection [25].

There are limitations to our findings. First, we did not prespecify the patient subgroup or composite outcome of OD and serious non-AIDS events in the original SMART analysis plan. Thus, our findings generate new hypotheses that require confirmation. Predefined components of our composite outcome have been reported for all SMART participants [14, 26]. For each end point, there is an excess risk in the DC compared with the VS group. The higher risk in DC compared with VS participants for this composite outcome is not limited to those in this small subgroup. The HR(DC/VS) for the composite of OD and serious non-AIDS events is >1 for each of the 4 cohorts classified by baseline ART use and is and significantly >1 for all SMART participants. Second, although the CIs around the HR estimates are wide as a result of the small number of composite events ($n = 27$), they are qualitatively similar to the reliably estimated 2-fold difference in risk for the overall SMART study. Third, we included a small number of participants who had prior AIDS-defining illnesses (23 DC and 29 VS participants). However, analyses that excluded these participants were consistent with the present results (table 3). Finally, in SMART we did not formally collect information on treatment-limiting toxicities that did not attain the severity to qualify as a grade 4 adverse events. During the period of observation, there were 3 DC participants (1%) and 24 VS participants (10%) who ceased ART with toxicities being the reason cited. During follow-up, there were 25 and 18 grade 4 events in the DC and VS groups, respectively.

These data indicate that the absolute risk of serious non-AIDS events in untreated patients is greater than the risk of AIDS at high $CD4^+$ cell counts. They reveal that risk reductions for AIDS associated with the earlier use of ART appear to be greater than previously estimated. Furthermore, the data suggest that reduced risk for serious non-AIDS outcomes arising from the early use of ART might substantially outweigh any increased risk of serious non-AIDS outcomes associated with the use of ART. We believe the data presented here provide a strong clinical rationale for the design and conduct of a randomized trial to estimate precisely the risks and benefits of the initiation of ART before the $CD4^+$ cell count declines to <350 cells/μL for the treatment of chronic HIV disease.

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Annals of Internal Medicine | ARTICLE

When to Start Antiretroviral Therapy in Resource-Limited Settings

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Background: The results of international clinical trials that are assessing when to initiate antiretroviral therapy (ART) will not be available for several years.

Objective: To inform HIV treatment decisions about the optimal CD4 threshold at which to initiate ART in South Africa while awaiting the results of these trials.

Design: Cost-effectiveness analysis by using a computer simulation model of HIV disease.

Data Sources: Published data from randomized trials and observational cohorts in South Africa.

Target Population: HIV-infected patients in South Africa.

Time Horizon: 5-year and lifetime.

Perspective: Modified societal.

Intervention: No treatment, ART initiated at a CD4 count less than 0.250×10^9 cells/L, and ART initiated at a CD4 count less than 0.350×10^9 cells/L.

Outcome Measures: Morbidity, mortality, life expectancy, medical costs, and cost-effectiveness.

Results of Base-Case Analysis: If 10% to 100% of HIV-infected patients are identified and linked to care, a CD4 count threshold for ART initiation of 0.350×10^9 cells/L would reduce severe opportunistic diseases by 22 000 to 221 000 and deaths by 25 000 to 253 000 during the next 5 years compared with ART initiation at 0.250×10^9 cells/L; cost increases would range from $142 million (10%) to $1.4 billion (100%). Either ART initiation strategy would increase long-term survival by at least 7.9 years, with a mean per-person life expectancy of 3.8 years with no ART and 12.5 years with an initiation threshold of 0.350×10^9 cells/L. Compared with an initiation threshold of 0.250×10^9 cells/L, a threshold of 0.350×10^9 cells/L has an incremental cost-effectiveness ratio of $1200 per year of life saved.

Results of Sensitivity Analysis: Initiating ART at a CD4 count less than 0.350×10^9 cells/L would remain cost-effective over the next 5 years even if the probability that the trial would demonstrate the superiority of earlier therapy is as low as 17%.

Limitation: This model does not consider the possible benefits of initiating ART at a CD4 count greater than 0.350×10^9 cells/L or of reduced HIV transmission.

Conclusion: Earlier initiation of ART in South Africa will probably reduce morbidity and mortality, improve long-term survival, and be cost-effective. While awaiting trial results, treatment guidelines should be liberalized to allow initiation at CD4 counts less than 0.350×10^9 cells/L, earlier than is currently recommended.

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Recent data from cohort studies and mathematical models in the developed world suggest that treatment outcomes of HIV-infected patients improve when antiretroviral therapy (ART) is initiated at CD4 counts less than 0.350×10^9 cells/L or even 0.500×10^9 cells/L (1–4). The question of when to start ART in HIV-infected patients is even more critical in resource-limited settings, in the context of higher rates of mortality and opportunistic diseases—including tuberculosis and other severe bacterial infections—at CD4 counts greater than 0.200×10^9 cells/L (5). At CD4 counts between 0.200 and 0.350×10^9 cells/L, the rates of such opportunistic diseases in South Africa may be 10-fold higher than those seen in the United States (5, 6). Several international clinical trials, including one in South Africa, are currently enrolling patients. These trials will explicitly address the clinical benefits of earlier ART initiation (at CD4 counts $<0.350 \times 10^9$ cells/L or $<0.500 \times 10^9$ cells/L) compared with the current World Health Organization (WHO) standard of care (stage 3 or 4 disease or when CD4 counts decrease to $<0.200 \times 10^9$ cells/L) (7–9).

Although clinical trials may provide insight into the optimal timing of ART in resource-limited settings, they can only address short-term outcomes and will not be available to inform practice for several years (8, 9). Our objective is to inform crucial decisions now, until these trials are reported, by using a model-based analysis to examine treatment strategies with different ART initiation thresholds in South Africa.

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Context

Rapid control of HIV infection is especially important in resource-limited countries with high rates of opportunistic infections. Therapy initiation guidelines would be useful for South African physicians while awaiting the results of ongoing trials of different CD4 count thresholds for therapy initiation.

Content

The investigators used a computer simulation of HIV disease to perform a cost-effectiveness analysis of 3 options: no treatment and thresholds of 0.250 and 0.350 $\times 10^9$ cells/L. Compared with a threshold of 0.250 $\times 10^9$ cells/L, starting at 0.350 $\times 10^9$ cells/L was highly cost-effective, even when the probability that a trial would show its superiority was as low as 17%.

Implication

Earlier antiretroviral therapy will probably prove to be superior in South Africa.

—The Editors

METHODS

Analytic Overview

Treatment Strategies

Using a computer-based model of HIV disease, we examined the policy decision regarding when to initiate ART in South Africa. We considered 3 strategies for interim treatment until the results of the ART initiation trials are available: no treatment (for comparison purposes), initiate ART at a CD4 count less than 0.250 $\times 10^9$ cells/L (or severe opportunistic disease) (7), and initiate ART at a CD4 count less than 0.350 $\times 10^9$ cells/L (or severe opportunistic disease). We initiated cotrimoxazole prophylaxis at a CD4 count less than 0.500 $\times 10^9$ cells/L in all strategies, in accordance with WHO recommendations (10). We examined the effect of this decision over both short-term (5-year) and lifetime horizons. We emphasize that all of these strategies would involve acting optimally on the results of the trials once they are available in 5 years.

To report on cost-effectiveness, we adopted a modified societal perspective, considering only direct, HIV-associated use of medical resources. We reported all costs in 2006 U.S. dollars by using country-specific gross domestic product (GDP) deflators and the 2006 mean exchange rate between the South African rand and the U.S. dollar (6.8 rand = 1 U.S. dollar) (11, 12). We discounted all costs and life expectancies at 3% per year (13). The WHO guidelines designate health interventions as cost-effective if the cost per quality-adjusted life-year is less than 3 times the country's per-capita GDP, and very cost-effective if the cost per quality-adjusted life-year is less than the country's per-capita GDP (14, 15). Although our analysis computes cost-effectiveness ratios in terms of years of life saved (YLS) (rather than quality-adjusted life-years), these thresholds provide general guidance. As a reference point, we compared the results with South Africa's 2006 per-capita GDP (U.S. $5400) (11).

Projections Over the Next 5 Years

We first examined the policy over the next 5 years to inform decisions regarding whether it would be best to consider therapy at a CD4 count threshold of 0.350 $\times 10^9$ cells/L rather than 0.250 $\times 10^9$ cells/L while waiting for the clinical trial results. We did this in 2 steps. First, we projected the number of South African patients who would require ART over the 5-year time horizon and their anticipated short-term clinical outcomes (defined as deaths and opportunistic diseases) and costs under alternative ART initiation scenarios. To do so, we used model-based methods, similar to those previously described (16), to examine how many HIV-infected persons in South Africa would be eligible for ART at a CD4 count threshold of 0.350 $\times 10^9$ cells/L versus 0.250 $\times 10^9$ cells/L over the 5-year time horizon. This estimate assumes steady HIV incidence over the next 5 years and accounts for HIV- and non–HIV-related deaths that occur before reaching the 0.350 $\times 10^9$ cells/L threshold.

By combining data from the WHO and the President's Emergency Plan for AIDS Relief, we estimated the proportion of HIV-infected persons who were identified and linked to care in South Africa (**Appendix 2**, available at www.annals.org) (17, 18). We estimated the effect if 10% (the proportion estimated to be receiving ART), 30% (the estimate of those receiving either ART or other, general President's Emergency Plan for AIDS Relief services), or 100% (as an upper bound) of patients were linked to care.

Finally, we assumed that a clinical trial will provide perfect information in 5 years about whether using an ART initiation threshold of 0.350 $\times 10^9$ cells/L is more efficacious than the current standard of care and developed a decision criterion under which it would be cost-effective ($<3 \times$ GDP) to invoke a policy of initiation at a CD4 count threshold of 0.350 $\times 10^9$ cells/L now while awaiting clinical trial results. This criterion included a threshold value for the probability that the trials will demonstrate the superiority of starting ART at CD4 counts less than 0.350 $\times 10^9$ cells/L.

Developing a Decision Criterion

To develop a decision criterion for earlier ART initiation now, we examined 2 potential policy scenarios (ART initiation at CD4 counts $<0.350 \times 10^9$ cells/L vs. $<0.250 \times 10^9$ cells/L) over the next 5 years and their associated clinical and economic outcomes (**Figure 1**). These outcomes excluded any long-term benefits, detriments, or costs potentially associated with either decision beyond the 5-year horizon. Although the calculated outcomes included ART-related toxicities, they also excluded any excess tox-

icity that might be associated with earlier ART beyond the 5-year horizon. If ART is initiated at a CD4 count less than 0.350×10^9 cells/L, the trial may demonstrate in 5 years that a 0.350×10^9 cells/L initiation threshold provides a benefit (probability P) or that it produces equivalent outcomes to a 0.250×10^9 cells/L threshold (probability $1 - P$). In the latter case, the associated costs of a 0.350×10^9 cells/L initiation threshold include not only those of earlier initiation but also the HIV medical costs accrued because of the additional deaths ($536 each) and opportunistic diseases (ranging from $105 to $1006 each) anticipated with using a CD4 count threshold of 0.250×10^9 cells/L compared with those anticipated with using 0.350×10^9 cells/L (19). If ART is initiated at a CD4 count less than 0.250×10^9 cells/L over the next 5 years, clinical outcomes and costs would be those derived for the short-term strategy of initiating ART at CD4 counts less than 0.250×10^9 cells/L. Averaging out the simple tree in **Figure 1**, we created the decision rule under which it would be economically efficient to set the initiation threshold at 0.350×10^9 cells/L now. We defined this decision rule by examining alternative values for P and using the cost-effectiveness willingness-to-pay threshold of 3 times GDP ($16 200/YLS).

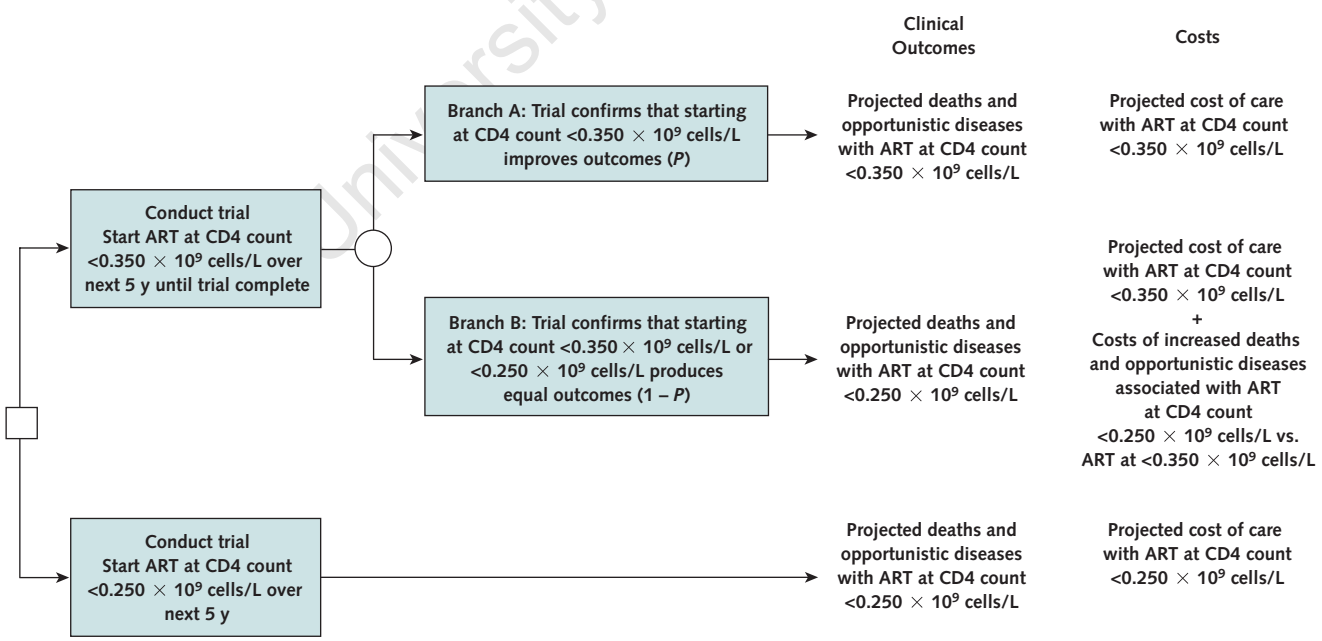
Lifetime Projections

After projecting 5-year outcomes, we then projected and compared the per-person life expectancy and mean lifetime HIV treatment costs for patients at CD4 count thresholds for ART initiation of 0.350×10^9 cells/L and 0.250×10^9 cells/L. We used these outcomes to produce incremental cost-effectiveness ratios; we used sensitivity analyses to examine the effect of key input parameters on the cost-effectiveness results.

CEPAC International Model

The CEPAC (Cost-Effectiveness of Preventing AIDS Complications) International model is a state-transition model of HIV disease in resource-limited settings, with data derived for several country-specific analyses, including South Africa (16, 20, 21). In brief, a cohort of hypothetical patients pass one at a time through health states, in monthly cycles, from entry into HIV care until death. Health states are defined to be both clinically and economically relevant and are stratified by current CD4 count, current HIV RNA level, and history of opportunistic disease. Opportunistic diseases are categorized into the following groups on the basis of cause, severity, and similarities in prophylaxis and treatment: mild or severe bacterial infections, mild or severe fungal infections, tuberculosis, toxoplasmosis, nontuberculous mycobacteriosis, *Pneumocystis jiroveci* pneumonia, and other mild and severe

Figure 1. **Decision tree that outlines ART strategy options over the next 5 years while trial results are pending.**



The payoffs in terms of both clinical outcomes and costs are delineated to the right of the tree. The probability P represents the chance that the forthcoming trials studying ART initiation will demonstrate a clinical benefit of ART initiation at a CD4 count threshold of 0.350×10^9 cells/L. Using a cost-effectiveness willingness-to-pay threshold of 3 times the per-capita gross domestic product of South Africa ($16,200/year of life saved), the tree suggests that initiating ART at CD4 counts less than 0.350×10^9 cells/L now would be optimal for values of P such that $16 200 $\geq$ {[$P \times$ costs (left branch)] + [$(1 - P) \times$ costs (middle branch)] – [costs (right branch)]} $\div$ {[$P \times$ outcomes (left branch)] + [$(1 - P) \times$ outcomes (middle branch)] – [outcomes (right branch)]}. As described in the Results section, values of P greater than 0.17 satisfy this decision rule. ART = antiretroviral therapy.

Table 1. **Model Input Parameters**

Variable	Base-Case Value	Reference
Baseline cohort characteristics		
Mean age (SD), *y*	32.8 (9)	5
Men, %	55	5
Mean CD4 count (SD), × 10^9 *cells/L*		
5-year projections for prevalent cohort	0.321 (0.146)	16
5-year projections for incident cohort	0.534 (0.164)	16
Long-term projections	0.375 (0.10)	Assumption
HIV RNA distribution, %		28
>100 000 copies/mL	42.5	
30 001–100 000 copies/mL	28.3	
10 001–30 000 copies/mL	17.9	
3001–10 000 copies/mL	7.8	
501–3000 copies/mL	2.3	
<500 copies/mL	1.2	
Natural history of disease		
Monthly CD4 count decrease, × 10^9 *cells/L*		30
>30 000 copies/mL	6.4	
10 001–30 000 copies/mL	5.4	
3001–10 000 copies/mL	4.6	
501–3000 copies/mL	3.7	
≤500 copies/mL	3.0	
Monthly risk for severe opportunistic disease, %*		5
Bacterial	0.08–0.71	
Fungal	0.02–2.22	
Tuberculosis	0.21–1.96	
Toxoplasmosis	0.00–0.06	
Nontuberculous mycobacteriosis	0.00–0.30	
Pneumocystis jiroveci pneumonia	0.00–0.12	
Other World Heath Organization stage 4–defining diseases	0.25–2.57	
Monthly risk for mild opportunistic disease, %*		5
Fungal	0.59–3.51	
Other	2.51–3.11	
Monthly risk for HIV-related death, %*		5
With no history of opportunistic disease	0.09–3.33	
With history of opportunistic disease	0.29–7.94	
Efficacy of antiretroviral therapy†		
First-line therapy‡	84%	23
Second-line therapy§	71%	24
Efficacy of cotrimoxazole, %‖		
Bacterial, severe	49.8	31, 32
Toxoplasmosis	83.3	31, 32
Pneumocystis jiroveci pneumonia	97.3	20
Costs, $¶		
First-line ART‡, monthly	24	36
Second-line ART§, monthly	47	36
Cotrimoxazole, monthly	1	34
Routine care, monthly**	10–129	5, 19, 33
Inpatient hospital care, per day	221	19
Outpatient clinic care, per visit	11	19
CD4 count, per test	10	35
HIV RNA, per test	49	35

ART = antiretroviral therapy.
* Range by CD4 count. Risk for opportunistic disease varies by CD4 stratum, divided into <0.050 × 10^9 cells/L, 0.051–0.200 × 10^9 cells/L, 0.201–0.350 × 10^9 cells/L, 0.351–0.500 × 10^9 cells/L, and >0.500 × 10^9 cells/L.
† HIV RNA suppression rate at 48 weeks.
‡ Nonnucleoside reverse transcriptase inhibitor–based regimen.
§ Protease inhibitor–based regimen.
‖ Reduction in probability of occurrence.
¶ In 2006 U.S. dollars.
** Range by CD4 count.

diseases (5). Deaths in the model occur from acute opportunistic events (within 30 days of the event), chronic AIDS (not within 30 days of an opportunistic disease), or non–HIV-related causes (22).

Effective ART in the model functions to suppress HIV RNA level and increase CD4 count (23, 24). Beyond the beneficial effect of increased CD4 count on opportunistic diseases and chronic HIV-related death (5), ART results in an additional reduction in opportunistic diseases and chronic HIV-related death, as investigators in Côte d'Ivoire and the United States recently reported (25, 26). Clinical assessments are assumed to occur every 3 months and CD4 and HIV RNA testing every 6 months while receiving therapy, consistent with South African recommendations (27). In accordance with the current standard of care, the model uses 2 sequential lines of ART; the second line is initiated when observed CD4 count decreases by 30% from its peak observed on-treatment level or when a severe opportunistic disease is observed at least 6 months after initiation of therapy (27). In accordance with current treatment guidelines, the second regimen for each patient is continued until death (7, 28).

Input Parameters

Trial-Eligible Patients

For the short-term projections, we developed a hypothetical cohort of HIV-infected patients who had the appropriate clinical attributes (**Table 1**). We defined both a prevalent HIV-infected cohort (to indicate those currently infected) and an incident HIV-infected cohort (to indicate those who will become infected and trial-eligible during the 5-year horizon) (see **Appendix 2**, available at www.annals.org). We used previously described methods (16) to estimate the characteristics (CD4 count and viral load distribution) of the prevalent cohort in South Africa that might be eligible now at a CD4 count threshold for ART initiation of 0.350 × 10^9 cells/L. The mean CD4 count was 0.321 × 10^9 cells/L (SD, 0.146) for the prevalent cohort; 21% of patients in this cohort would be eligible for ART initiation at a threshold of 0.350 × 10^9 cells/L versus 0.250 × 10^9 cells/L. We also used projections from the Actuarial Society of South Africa to forecast the number of incident HIV infections anticipated over the 5-year trial horizon (29). The incident cohort had a mean CD4 count of 0.534 × 10^9 cells/L (SD, 0.164). In the first year after incident infection, 15% of patients in this cohort would be eligible for ART initiation at a threshold of 0.350 × 10^9 cells/L versus 0.250 × 10^9 cells/L.

Because the CD4 counts and HIV RNA distributions of patients in the prevalent cohort and the incident cohorts differ, we derived survival data separately for each cohort. Using the CEPAC International model, we initialized the prevalent and incident cohorts to create a composite picture of the CD4 and HIV RNA distribution of each cohort, given their duration of infection (16). We projected

the survival for HIV-infected patients who did not receive ART (for the no ART comparison) and those who began receiving ART at counts less than 0.350×10^9 cells/L and less than 0.250×10^9 cells/L (16). We calculated annual probabilities of survival (conditional on survival to the beginning of the year) by dividing the number of HIV-infected patients who were alive at the end of a given calendar year by the number who were alive at the end of the previous year. This reflects a patient's probability of surviving through the year, given that the patient was alive at the beginning of the year.

Cohort Characteristics

For the long-term projections, the simulated cohort was designed to resemble the characteristics of HIV-infected persons in South Africa. Clinical and demographic characteristics were based on data from the Cape Town AIDS Cohort (5). In the absence of specific data from South Africa, we obtained rates of CD4 count decrease, stratified by baseline HIV RNA level, from the Multicenter AIDS Cohort Study in the United States (**Table 1**) (30). We assumed that patients who entered the model were initiating HIV care and had a mean age of 32.8 years. For the long-term projections, we used a mean baseline CD4 count of 0.375×10^9 cells/L to simulate enrollment criteria for the ART initiation trials; 42.5% of patients had baseline HIV RNA levels greater than 100 000 copies/mL (**Table 1**).

Opportunistic Disease Prophylaxis and Efficacy of Antiretroviral Therapy

In the absence of reported data from South Africa, we derived the efficacy of cotrimoxazole prophylaxis in our model from clinical trials in Côte d'Ivoire (31, 32). In Côte d'Ivoire, cotrimoxazole confers protection against bacterial infections, *P. jiroveci* pneumonia, isospora and malaria, and toxoplasmosis; isospora infection and malaria are not reported in the South African data (**Table 1**) (20). We assumed that 2 sequential antiretroviral regimens would be available. First-line therapy was a nonnucleoside reverse transcriptase inhibitor–based regimen with which a reported 84% of patients experienced HIV RNA suppression at 48 weeks (mean CD4 count increase, 0.184×10^9 cells/L [interquartile range, 0.108 to 0.271×10^9 cells/L]) (23). Patients for whom the first-line regimen failed received a protease inhibitor–based second-line regimen. In this regimen, we incorporated the need for recycled nucleoside reverse transcriptase inhibitors, with a published estimate of 71% of patients experiencing HIV RNA suppression at 48 weeks (mean CD4 count increase, 0.151×10^9 cells/L [interquartile range, 0.105 to 0.239×10^9 cells/L]) (24).

Costs

We considered HIV-associated direct medical resource use, including inpatient days, outpatient visits, laboratory tests, and medication costs (19, 33–37). We excluded direct nonmedical costs and indirect costs (such as patient time and lost wages). We used a utilization analysis and unit costing approach to derive health care use from the Cape Town AIDS cohort (5, 19, 33). We derived costs on the basis of the number of inpatient hospital days and outpatient clinic visits associated with each type of opportunistic disease, or each month of routine HIV care in the absence of opportunistic disease, and the number of days and visits during the month of death.

Role of the Funding Source

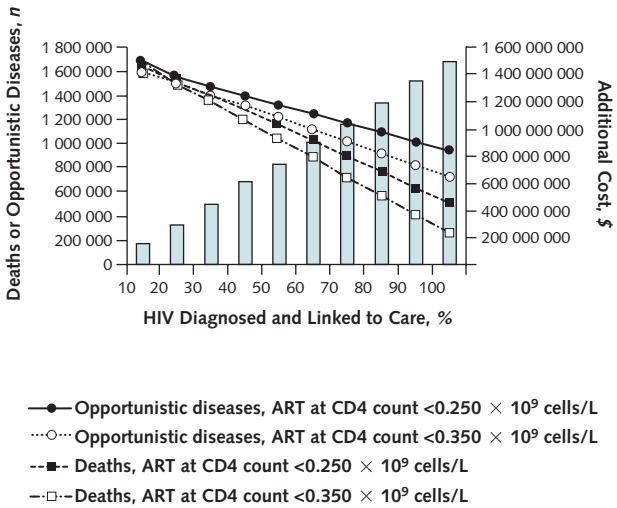
The study was funded by the National Institute of Allergy and Infectious Diseases and the Doris Duke Foundation. The funding sources had no input in the study design, analysis and interpretation of data, writing of the report, or decision to submit for publication.

Table 2. **Clinical and Economic Outcomes of Both ART Initiation Strategies Over the Next 5 Years**

Scenario and ART Initiation Criteria	Opportunistic Diseases, *n*	Deaths, *n*	Discounted Costs, $
At 10% HIV case identification and linkage to care			
CD4 count $<0.350 \times 10^9$ cells/L or opportunistic disease	1 599 859	1 664 458	9 974 640 200
CD4 count $<0.250 \times 10^9$ cells/L or opportunistic disease	1 621 969	1 689 739	9 832 663 100
Difference*	(22 110)	(25 281)	141 977 100
At 30% HIV case identification and linkage to care			
CD4 count $<0.350 \times 10^9$ cells/L or opportunistic disease	1 406 618	1 348 856	10 436 784 100
CD4 count $<0.250 \times 10^9$ cells/L or opportunistic disease	1 472 947	1 424 699	10 010 852 800
Difference*	(66 329)	(75 843)	425 931 300
At 100% HIV case identification and linkage to care			
CD4 count $<0.350 \times 10^9$ cells/L or opportunistic disease	730 272	244 249	12 054 287 800
CD4 count $<0.250 \times 10^9$ cells/L or opportunistic disease	951 370	497 059	10 634 516 900
Difference*	(221 097)	(252 810)	1 419 770 900

* Results at CD4 count threshold of 0.350×10^9 cells/L − results at 0.250×10^9 cells/L. Data in parentheses denote fewer opportunistic diseases and deaths with ART initiation at $<0.350 \times 10^9$ cells/L. ART = antiretroviral therapy.

Figure 2. **Model-based projections over the next 5 years for strategies of ART initiation at CD4 count thresholds of 0.350 × 10^9 cells/L and 0.250 × 10^9 cells/L.**



Squares indicate total deaths and circles indicate total opportunistic diseases for the 2 strategies (left vertical axis). The bars indicate excess total costs of initiating ART at a threshold of 0.350 × 10^9 cells/L compared with 0.250 × 10^9 cells/L over a 5-year horizon (right vertical axis). The horizontal axis represents results at varying proportions of HIV cases identified and linked to care in the population. ART = antiretroviral therapy.

RESULTS

Outcomes Projected Over a 5-Year Horizon

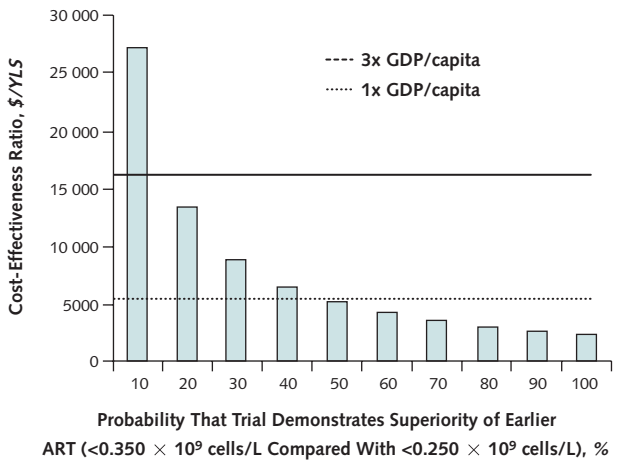
We estimated that 4.7 million HIV-infected persons in South Africa would become eligible to start ART if the CD4 count threshold is 0.350 × 10^9 cells/L instead of 0.250 × 10^9 cells/L over a 5-year time horizon. Among such persons, 1.2 million are eligible now, 1.6 million will be eligible over the next year, and 1.9 million will become eligible over the ensuing 3 years.

For our assumptions that 10%, 30%, and 100% of these 4.7 million persons are identified and linked to care, we projected the opportunistic diseases, deaths, and costs over the next 5 years of alternative ART strategies while awaiting results of the ART initiation trials (**Table 2**). At the conservative HIV identification and linkage to care estimate of 10%, a CD4 count threshold of 0.350 × 10^9 cells/L for ART initiation would result in fewer total cases of opportunistic disease (1 599 900 vs. 1 622 000) and fewer total deaths (1 664 500 vs. 1 689 700) than a threshold of 0.250 × 10^9 cells/L. A threshold of 0.350 × 10^9 cells/L would also lead to a discounted $142 million cost increase over the next 5 years, which reflects the additional treatment costs (offset in part by the reduced incidence of opportunistic diseases). At the maximum estimate (100% identification and linkage to care), 221 000 cases of opportunistic disease and 253 000 deaths could be averted. In this situation, the additional costs of using a threshold of 0.350 × 10^9 cells/L would exceed $1.4 billion. **Figure 2** provides results for the clinical and economic effect if between 10% and 100% of HIV-infected, eligible patients are identified and present for care. Clinical and cost results move together—the fewer patients identified, the fewer deaths and opportunistic diseases are averted and the lower the added total costs of an earlier ART initiation strategy.

Decision Criteria

We then examined the probability that the data from the forthcoming trials would provide enough statistical evidence that a CD4 count threshold for ART initiation of 0.350 × 10^9 cells/L is superior to a 0.250 × 10^9 cells/L threshold—which would confirm the model-based results. If this is certain (100% probability), the incremental cost-effectiveness of a threshold of 0.350 × 10^9 cells/L compared with 0.250 × 10^9 cells/L is $2400/YLS, considering only the costs and benefits over the next 5 years. If the probability that the trials show a benefit to a threshold of 0.350 × 10^9 cells/L decrease to 10%, the incremental cost-effectiveness over the next 5 years would increase to $27 100/YLS. Using the established WHO cost-effectiveness guideline, a policy option to initiate ART at CD4 counts less than 0.350 × 10^9 cells/L should be used over the next 5 years if the probability that the trial will confirm model-based results is 17% or greater (**Figure 3**). In sensitivity analyses, we varied the composite increase in deaths asso-

Figure 3. **Incremental cost-effectiveness of ART at CD4 count thresholds of 0.350 × 10^9 cells/L versus 0.250 × 10^9 cells/L at alternative probability values.**



P represents the probability that the trial will confirm model-based results indicating a benefit of earlier therapy (see Methods and **Figure 1**). The incremental cost-effectiveness is provided for the 5-year time horizon. The height of the bar provides the cost-effectiveness ratio of an initiation threshold of 0.350 × 10^9 cells/L versus 0.250 × 10^9 cells/L for alternative values of *P*; bars that remain below the horizontal solid line (<3 × GDP) are considered to be cost-effective and those that remain below the horizontal dotted line (<1 × GDP) are considered to be very cost-effective. ART = antiretroviral therapy; GDP = gross domestic product in South Africa (U.S. $5400); YLS = year of life saved.

Table 3. **Life Expectancy, Cost, and Cost-Effectiveness of Strategies for HIV Care**

Strategy	Discounted Per-Person Lifetime Cost, $*	Discounted (Undiscounted) Per-Person Life Expectancy, y*	Incremental Cost-Effectiveness Ratio, $/YLS†
No treatment	3930	3.83 (4.11)	–
Initiating ART at CD4 counts <0.250 × 10^9 cells/L or opportunistic disease	12 730	11.71 (15.23)	1100
Initiating ART at CD4 counts <0.350 × 10^9 cells/L or opportunistic disease	13 620	12.48 (16.27)	1200

ART = antiretroviral therapy; YLS = year of life saved.
* Discounted at 3% per year.
† Incremental cost-effectiveness ratios are calculated compared with the next less costly strategy. Because of rounding, the incremental cost-effectiveness ratios may not match exactly the ratios of lifetime costs and projected survival reported in the table.

ciated with an ART initiation threshold of 0.250×10^9 cells/L versus 0.350×10^9 cells/L from 2-fold more deaths (base case) to 1.5-fold more deaths. The decreased benefits of the 0.350×10^9 cells/L threshold can simulate situations in which earlier therapy is less effective on an individual level than the model projects or linkage to care is lower at a threshold of 0.350×10^9 cells/L than at 0.250×10^9 cells/L because of the absence of clinical trial data. Under such a scenario, a policy option to initiate ART at CD4 counts less than 0.350×10^9 cells/L should be used over the next 5 years if the probability that the trial will confirm model-based results is 28% or greater (**Appendix Figure**, available at www.annals.org).

Lifetime Projections

When we projected long-term outcomes for a cohort with a mean CD4 count of 0.375×10^9 cells/L, the no-treatment strategy resulted in a mean survival of 3.83 years (4.11 undiscounted), compared with 11.71 years (15.23 undiscounted) at a CD4 count threshold for ART initiation of 0.250×10^9 cells/L and 12.48 years (16.27 undiscounted) at a threshold of 0.350×10^9 cells/L (**Table 3**). The survival curves that corresponded to ART initiation thresholds of 0.350×10^9 cells/L and 0.250×10^9 cells/L diverged within about 1 year, after which time they became essentially parallel, when nearly all patients had initiated ART at a threshold of 0.250×10^9 cells/L; by year 3, initiation at CD4 counts less than 0.350×10^9 cells/L maintained a consistent 6% absolute advantage in the proportion of the cohort alive through year 10 (**Figure 4**). Per-person lifetime direct costs were lowest with no ART ($3930) (**Table 3**). Lifetime costs increased to $12 730 per person at an initiation threshold of 0.250×10^9 cells/L and $13 620 at 0.350×10^9 cells/L. The incremental cost-effectiveness ratio was $1100/YLS at a CD4 count threshold of 0.250×10^9 cells/L compared with no treatment and $1200/YLS at a threshold of 0.350×10^9 cells/L compared with 0.250×10^9 cells/L (**Table 3**).

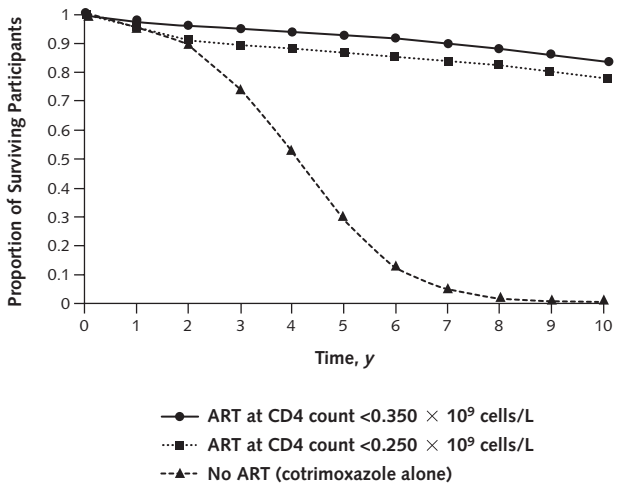
Sensitivity Analyses on Lifetime Projections

Because the long-term results consistently favored a CD4 count threshold for ART initiation of 0.350×10^9 cells/L, we designed the sensitivity analyses to bias against earlier initiation. Specifically, we examined large decrements in second-line antiretroviral efficacy in the earlier therapy strategies. Second-line efficacy would have to be fewer than 39% of patients achieving HIV RNA suppression at 48 weeks—a 32% relative decrease from the base case—to match the projected survival rate from using a threshold of 0.250×10^9 cells/L. To examine the effect of pill fatigue and failed retention in care (38, 39), we also considered higher rates of discontinuation of care. We assumed that some patients who started ART at CD4 counts less than 0.350×10^9 cells/L discontinued ART at the time they would have started receiving second-line regimens, thereby only realizing the benefits of first-line therapy (although they still received prophylaxis and treatment for opportunistic diseases). More than 19% of patients who received ART at a threshold of 0.350×10^9 cells/L at the time of treatment failure would need to discontinue treatment to decrease survival to that associated with a threshold of 0.250×10^9 cells/L. Finally, when we included a hypothetical third-line antiretroviral regimen, survival and costs increased for both ART treatment strategies. The incremental cost-effectiveness of using a threshold of 0.350×10^9 cells/L was largely unchanged from that of using 0.250×10^9 cells/L ($1000/YLS). **Appendix 2** (available at www.annals.org) provides detailed results of these and other analyses.

DISCUSSION

Although the forthcoming trials in South Africa and other resource-limited settings should yield important information in the next 5 to 10 years, our analysis suggests that, until trial data are available, a CD4 count threshold of 0.350×10^9 cells/L for initiating ART would probably yield better clinical outcomes than a lower threshold. The magnitude of such benefits multiply with increased rates of HIV identification and linkage to care. A threshold of 0.350×10^9 cells/L is also expected to be highly cost-effective in the interim. Many of the clinical benefits of starting earlier occur beyond the 5-year time horizon of the trial (manifested in increased life expectancy). Even so, our results suggest that a threshold of 0.350×10^9 cells/L re-

Figure 4. **Model-generated survival curves for ART.**



The annual mortality hazard 2 years after entry into care was 0.01 for a threshold of 0.350×10^9 cells/L, 0.05 for 0.250×10^9 cells/L, and 0.06 for no ART. Two years after entry into care, the composite annual hazard of severe opportunistic disease, tuberculosis, or death was 0.06 for a threshold of 0.350×10^9 cells/L, 0.16 for 0.250×10^9 cells/L, and 0.17 for no ART (data not shown). ART = antiretroviral therapy.

mains cost-effective if the probability is 17% or greater that the forthcoming trials will demonstrate improved clinical outcomes with starting ART earlier.

When ART is initiated according to current treatment guidelines, we found that an ART initiation threshold of 0.250×10^9 cells/L is very cost-effective in the long term for HIV infection in South Africa, with a ratio of $1100/YLS (7); for a threshold of 0.350×10^9 cells/L, the cost-effectiveness ratio is $1200/YLS. That these ratios are similar suggests that if HIV treatment is worth initiating, early initiation provides similar value to later treatment. We specifically designed sensitivity analyses to see how these results might change and found that very high rates of drug resistance and pill fatigue would be required to make earlier therapy not cost-effective. Because our results depend heavily on the frequency of opportunistic diseases at higher CD4 counts, morbidity rates should be assessed carefully at high CD4 counts; initiation of therapy at a greater CD4 count threshold than 0.350×10^9 cells/L may be justified in South Africa.

Conducting the current trials remains critically important in informing the question of when to start ART. Evidence-based guidelines continue to maintain that randomized, controlled trials are the gold standard for developing policy; modeling analyses are still considered lower levels of evidence (40, 41). As such, randomized trials will probably be used as the benchmark evidence for HIV treatment throughout the world. Meanwhile, our model-based analysis suggests that opening up the option to start ART earlier in the disease course would probably improve clinical outcomes, at least until trial results are available. Our results suggest that 25 000 lives may be at stake; waiting 5 years for trial results could be costly in human terms.

Despite findings that a CD4 count threshold for ART initiation of 0.350×10^9 cells/L may be beneficial, a study on patient characteristics at presentation to care in South Africa suggests that a discussion of earlier versus deferred ART initiation may not be germane at present; in that study, the mean CD4 count of patients who started ART was only 0.096×10^9 cells/L (42). However, wider implementation of the WHO guidelines for using HIV testing technologies (43) and improved HIV screening and linkage to care should result in the identification of more patients who are eligible for earlier therapy initiation (7). Decisions need to be made on how best to optimize their care, and efforts to identify them must continue if a policy of earlier therapy is to have a meaningful effect. Our analysis demonstrates that a CD4 count threshold of 0.350×10^9 cells/L is highly effective and confers similar value to a threshold of 0.250×10^9 cells/L.

However, an ART initiation threshold of 0.350×10^9 cells/L may not be optimal in some cases, such as when treatment capacity is limited—as is currently the case in many places (16). In such settings, prioritization—whether ART should be provided on a first-come, first-served basis or on a CD4 count–based policy—is already problematic (44). With inadequate treatment capacity, increasing the treatment initiation threshold for all patients to a CD4 count of 0.350×10^9 cells/L, without prioritization for the sickest patients, could result in more deaths in the near term, even if earlier therapy is associated with long-term benefits. Thus, guidelines that move toward ART initiation at higher CD4 counts should be implemented only in settings with adequate capacity to treat all of those who are eligible and at highest risk.

Our study has limitations. First, this analysis does not represent an assessment of the estimated value of perfect information, which would examine whether the trial is worth doing. Because trials are already enrolling, we address the question of the optimal clinical strategy while awaiting the results of those trials. Second, we incorporated input data from multiple sources. Although they were uniformly derived, not all data are from similar cohorts in South Africa. Sensitivity analyses demonstrate that within reasonable reported ranges, our major conclusions are robust to these data estimates. Third, implementation of ART strategies by CD4 threshold in international settings—where CD4 testing is not universally available—may require investments in infrastructure. Fourth, our model does not account for the potential benefits of ART, unrelated to opportunistic diseases, that might be attributable to treatment at CD4 thresholds higher than the current standard of care (45). We also do not capture any additional benefits in preventing HIV transmission that earlier ART may confer because of viral load reduction (46). To the extent that these benefits occur, earlier therapy would be even more advantageous. Finally, earlier therapy

may have other additional benefits in African countries that have higher rates of malaria and bacterial diseases than those documented in South Africa (5, 31).

Ongoing randomized trials are studying the question of when to initiate ART in resource-limited settings. As these trials continue to enroll and accrue follow-up toward the primary outcomes, decisions must be made now regarding the optimal ART initiation policy in these settings. While we await trial results in settings of adequate treatment capacity, this study demonstrates that it is probably both effective and cost-effective to liberalize the opportunity for ART to be initiated at a CD4 count threshold of 0.350×10^9 cells/L in South Africa.

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Reproducible Research Statement: We have provided a detailed technical appendix (**Appendix 2**, available at www.annals.org) for this and many other CEPAC papers. This appendix describes the depth of model structure and the breadth of the input parameters; for further questions, please contact Dr. Walensky.

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Appendix 1: CEPAC-International Members

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Appendix 2: Technical Appendix

We offer this technical appendix to provide supplementary data to reviewers regarding requested model input parameters and further sensitivity analyses.

Methods

Appendix Table 1 provides the calculations we used to project the number of persons eligible, by year, for the treatment decision of ART initiation at a CD4 count threshold of 0.350×10^9 cells/L versus 0.250×10^9 cells/L.

Appendix Table 2 provides estimates of HIV-infected persons who were identified and linked to care in 3 countries. These estimates are obtained in part from WHO country-specific reports on the number of persons living with HIV/AIDS (17) and from the President's Emergency Plan for AIDS Relief reports on the number of HIV-infected persons who received care and support in fiscal year 2008 as well as those who received ART (18).

Appendix Table 3 provides the monthly risk for opportunistic diseases, stratified by the CD4 cell counts used in the model (**Table 1** provides a summary of these data).

Results

Sensitivity Analysis of Trial Projections

Table 2 indicates an approximate 2-fold increase in total deaths at a CD4 count threshold for ART initiation of 0.250×10^9 cells/L compared with 0.350×10^9 cells/L over a 5-year horizon. Because this 2-fold estimate results from model output, many degrees of freedom within the model inform this estimate, including rates of opportunistic diseases, deaths associated with those events, ART efficacy, and AIDS-related mortality itself.

We examined the composite death rates of model output from the 2 ART strategies, diminishing the benefit of a CD4 count threshold for ART initiation of 0.350×10^9 cells/L from 2.0 to 1.1. For example, if the initial death rate in year 1 was 0.03 when using a threshold of 0.350×10^9 cells/L and 0.07 when using 0.250×10^9 cells/L, then our sensitivity analysis for a benefit of 1.5 examined a new death rate for year 1 of 0.05 for a threshold of 0.350×10^9 cells/L ($[(0.07 - 0.03) \times 0.5] + 0.03$). For each sensitivity analysis, we determined this adjusted death rate in every year of the 5-year horizon. We used a similar approach with our estimates of opportunistic diseases (decreasing the benefit of a 0.350×10^9 cells/L threshold by half) and used detailed model output to adjust the effect this would have on costs. For increasing benefits of a threshold of 0.350×10^9 cells/L (ranging from 1.0 [no benefit] to 2.0 [our results]), the

Appendix Figure provides alternative values for P that meet the following 2 criteria: the trial demonstrates superiority of an ART initiation threshold of 0.350×10^9 cells/L and the cost-effectiveness ratio (over 5 years) is less than \$16 200/YLS ($<3 \times$ GDP). The solid arrow indicates the threshold P value of 17%; at a value of 1.5 (half the benefit that our model suggests for a threshold of 0.350×10^9 cells/L), the value for P is 28%, indicated by the hollow arrow.

Sensitivity Analyses on Lifetime Projections

Because the results of the lifetime analyses consistently favored earlier therapy, we designed sensitivity analyses to bias against earlier initiation. Specifically, we examined large decrements in second-line antiretroviral efficacy in the 0.350×10^9 cells/L strategies; presumed poorer downstream adherence in the 0.350×10^9 cells/L strategies; and included a third-line antiretroviral regimen, which potentially decreased the survival benefit of initiating ART at 0.350×10^9 cells/L.

Efficacy and Cost of Antiretroviral Therapy

First-line therapy efficacy varied from 79% to 94% of patients achieving HIV RNA suppression at 48 weeks (base case, 84%), with minimal effect on the life expectancy and cost-effectiveness ratios of ART initiation at 0.350×10^9 cells/L compared with 0.250×10^9 cells/L (**Appendix Table 4**). Assuming that a third line of available antiretrovirals (at the same cost and efficacy as the second-line regimen) increased survival and costs in both the 0.350×10^9 cells/L and 0.250×10^9 cells/L strategies, the incremental cost-effectiveness of earlier therapy improved to \$1100/YLS compared with deferred therapy. Changes in the cost of antiretroviral drugs from 50% to 200% of the base case altered the cost-effectiveness ratio of earlier compared with deferred therapy from \$800 to 2400/YLS (**Appendix Table 4**).

Discontinuation of Antiretroviral Therapy

To examine the effect of pill fatigue and failed retention in care, we considered increasing rates of discontinuation of care. Specifically, we assumed that some patients discontinued antiretroviral therapy at the time they would have started receiving a different regimen, thereby only realizing the benefits of first-line therapy (although they still received prophylaxis and treatment for opportunistic diseases). More than 19% of patients who received therapy at a CD4 count threshold of 0.350×10^9 cells/L would need to discontinue treatment to decrease survival to that associated with deferred therapy. We also considered discontinuation of antiretroviral therapy at thresholds of 0.350 and 0.250×10^9 cells/L. If the rate of discontinuation at a threshold of 0.250×10^9 cells/L was similar to that at 0.350×10^9 cells/L, the benefits of earlier therapy diminished as the rates of discontinuation increased; for example, the increase in life expectancy associated with a threshold of 0.350×10^9 cells/L compared with 0.250×10^9 cells/L was 0.8 year with no discontinuation, 0.7 year with 20% discontinuation, and 0.5 year with 50% discontinuation.

Appendix Table 1. **Calculations Used to Determine the Eligible Cohort***

HIV Cases (Index Year)	Year	Persons With CD4 Count Between 0.250 and 0.350 × 10^9 cells/L	
		Proportion	Persons, *n*
5 628 474 (2008)†	2008	0.210	1 176 271
	2009	0.263	1 480 606
	2010	0.124	699 602
	2011	0.066	373 664
	2012	0.030	166 853
497 756 (2009)‡	2009	0.153	76 343
	2010	0.157	78 094
	2011	0.161	80 298
	2012	0.136	67 834
492 244 (2010)‡	2010	0.153	75 498
	2011	0.157	77 229
	2012	0.161	79 409
488 186 (2011)‡	2011	0.153	74 875
	2012	0.157	76 592
485 346 (2012)‡	2012	0.153	74 440

* Using methods described here and in reference 16.
† Prevalent cases.
‡ Incident cases.

Appendix Table 2. **Estimates of HIV-Infected Persons Identified and Linked to Care in 3 African Countries***

Country	Persons Estimated To Be Living With HIV/AIDS, *n*	Persons Receiving Care, *n* (%)	Persons Receiving ART, *n* (%)
South Africa	5 200 000	1 852 700 (34)	549 700 (10)
Côte d'Ivoire	605 000	103 200 (17)	50 500 (8)
Uganda	615 000	392 100 (63)	145 000 (24)

ART = antiretroviral therapy.
* We use the midpoint of the estimated range of cases from the World Health Organization to calculate percentages. The denominator is the estimated number of persons living with HIV/AIDS.

Appendix Table 3. Monthly Risk for Opportunistic Diseases, by CD4 Count

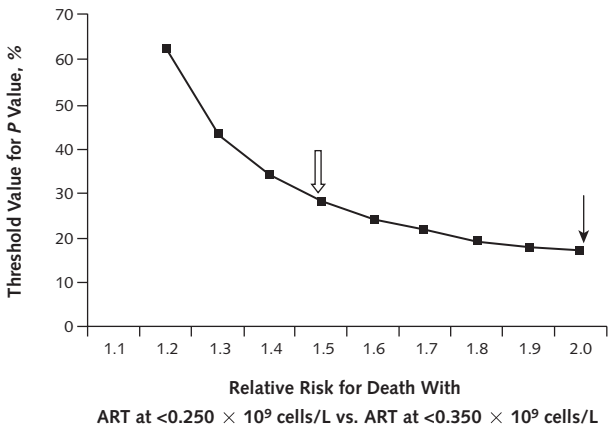
Disease Type	Risk, %			
	CD4 Count 0–0.050 × 10^9 cells/L	CD4 Count 0.051–0.200 × 10^9 cells/L	CD4 Count 0.201–0.350 × 10^9 cells/L	CD4 Count >0.350 × 10^9 cells/L
Severe opportunistic disease, %				
Bacterial	0.71	0.30	0.13	0.08
Fungal	2.22	0.42	0.10	0.02
Tuberculosis	1.96	1.14	0.67	0.21
Toxoplasmosis	0.06	0.02	0.00	0.00
Nontuberculous mycobacteriosis	0.30	0.03	0.00	0.01
Pneumocystis jiroveci pneumonia	0.12	0.02	0.00	0.01
Other World Health Organization stage 4–defining diseases	2.57	0.68	0.30	0.25
Mild opportunistic disease, %				
Fungal	3.51	2.05	1.28	0.59
Other	3.06	2.70	3.11	2.51

Appendix Table 4. Selected Sensitivity Analyses and Results for the Projected Cost-Effectiveness of Earlier ART Initiation

Model Input Parameter	Parameter Range	Range Observed in Model Output	
		Survival, *y**	Incremental Cost-Effectiveness, *$/YLS*†
Base case	–	12.48	1200
Efficacy of first-line ART	79%–94% achieving virologic suppression at 48 wk	12.17–12.63	1200–1100
Number of ART regimens available	1–3 lines	10.89–13.89	1100‡–1100‡
Cost of ART	0.5–2.0 × base-case value (first-line monthly cost range, $12–$48; second-line monthly cost range, $24–$94)	–	800‡–2400
ART switching rule	"Only switch at opportunistic disease event" to "switch at opportunistic disease event or 50% decrease in peak CD4 count"	12.47–12.47	1200–1200
ART stopping rule	"Stop at opportunistic disease event or 90% decrease in peak CD4 count" to "never stop"	10.68–12.48	500‡–1200
CD4-independent effect of ART on death and opportunistic diseases	No effect	8.96	1100

ART = antiretroviral therapy; YLS = year of life saved.
* Discounted.
† Unless otherwise noted, incremental cost-effectiveness ratios are reported for initiating ART at CD4 counts $<0.350 \times 10^9$ cells/L versus $<0.250 \times 10^9$ cells/L.
‡ We report the incremental cost-effectiveness ratio for initiating ART at CD4 counts $<350 \times 10^9$ cells/L compared with no treatment because we excluded all strategies that were dominated when we calculated cost-effectiveness ratios.

Appendix Figure. **Sensitivity analysis on the benefit of ART at a CD4 count less than 0.350 × 10^9 cells/L.**



Results from the model suggested a 2-fold decrease in the death rate with ART initiation at a CD4 count $<0.350 \times 10^9$ cells/L. The vertical axis shows the threshold *P* value at which the trial will demonstrate a benefit of ART initiation at $<0.350 \times 10^9$ cells/L and that also meets the cost-effectiveness threshold criterion of <$16 200 (3 times the gross domestic product of South Africa). The solid arrow indicates the 17% threshold discussed in the article; the open arrow indicates the results if the benefit of ART initiation at $<0.350 \times 10^9$ cells/L were half (1.5) of what the model projected. ART = antiretroviral therapy.

Initiating patients on antiretroviral therapy at CD4 cell counts above 200 cells/μl is associated with improved treatment outcomes in South Africa

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Objectives: To compare treatment outcomes by starting CD4 cell counts using data from the Comprehensive International Program of Research on AIDS-South Africa trial.

Design: An observational cohort study.

Methods: Patients presenting to primary care clinics with CD4 cell counts below 350 cells/μl were randomized to either doctor or nurse-managed HIV care and followed for at least 2 years after antiretroviral therapy (ART) initiation. Clinical and laboratory outcomes were compared by baseline CD4 cell counts.

Results: Eight hundred and twelve patients were followed for a median of 27.5 months and 36% initiated ART with a CD4 cell count above 200 cells/μl. Although 10% of patients failed virologically, the risk was nearly double among those with a CD4 cell count of 200 cells/μl or less vs. above 200 cells/μl (12.2 vs. 6.8%). Twenty-one deaths occurred, with a five-fold increased risk for the low CD4 cell count group (3.7 vs. 0.7%). After adjustment, those with a CD4 cell count of 200 cells/μl had twice the risk of death/virologic failure [hazard ratio 1.9; 95% confidence interval (CI), 1.1–3.3] and twice the risk of incident tuberculosis (hazard ratio 1.90; 95% CI, 0.89–4.04) as those above 200 cells/μl. Those with either a CD4 cell count of 200 cells/μl or less (hazard ratio 2.1; 95% CI, 1.2–3.8) or a WHO IV condition (hazard ratio 2.9; 95% CI, 0.93–8.8) alone had a two-to-three-fold increased risk of death/virologic failure vs. those with neither, but those with both conditions had a four-fold increased risk (hazard ratio 3.9; 95% CI, 1.9–8.1). We observed some decreased loss to follow-up among those initiating ART at less than 200 cells/μl (hazard ratio 0.79; 95% CI, 0.50–1.25).

Conclusion: Patients initiating ART with higher CD4 cell counts had reduced mortality, tuberculosis and less virologic failure than those initiated at lower CD4 cell counts. Our data support increasing CD4 cell count eligibility criteria for ART initiation.

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Keywords: CD4 cell count, highly active antiretroviral therapy, HIV, mortality, Sub-Saharan Africa, tuberculosis, virologic failure

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Introduction

With the increase in global funding for HIV/AIDS, the developing world has seen unprecedented access to lifesaving antiretroviral therapy (ART) over the past 5 years. Funds mandated toward rapidly scaling up access to ART have been successfully targeted and nearly four million people are now on ART [1]. When large-scale treatment programs began, in most cases, treatment was limited to patients with advanced disease. The ideal time to initiate ART is currently unknown [2]. Although guidelines for resource-rich environments currently recommend ART initiation at CD4 cell counts below 350 cells/μl [3,4], developing country guidelines recommended initiating ART at CD4 cell counts of 200 cells/μl or less in the absence of clinical disease until November 2009 when the WHO recommended initiating treatment at CD4 cell counts below 350 cells/μl [5,6]. As new evidence from resource-rich environments has accumulated, showing that starting ART at higher CD4 cell counts is associated with better treatment outcomes [7–9], programs in resource-limited settings, given limited resources, must make difficult choices about whether or not to raise initiating CD4 cell count thresholds to higher levels.

The debate about when to initiate treatment is difficult as ART is a lifelong treatment that has significant cost and can have significant side effects. On an individual level, decisions about when to initiate therapy must balance the potential medical benefits of initiating at higher CD4 cell counts [10] and reductions in HIV transmission [11] with the risk of toxicity and the costs associated with longer time on treatment. On a public health level, a decision about when to initiate ART must balance not only any expected population-level benefits of initiating treatment at higher CD4 cell counts with the cost implications of potentially increased demand for treatment if treatment thresholds are raised but also any possible cost savings associated with earlier treatment, including reduced hospitalization and treatment of opportunistic infections [12]. The first step toward rationalizing decisions on when to initiate ART is to assess, using data from resource-limited settings, the likely treatment benefits that can be expected.

Recent evidence from the developed world suggests that starting ART at CD4 cell counts below 350 cells/μl improves treatment outcomes and decreases mortality compared with waiting until the CD4 cell count drops below 200 cells/μl [13–17], and the benefits may even begin when initiating at CD4 cell counts above 350 cells/μl [13], yet limited evidence from the developing world exists to inform policy [18]. To date, only one study from a developing country has attempted to randomize patients to initiate ART at CD4 cell counts below 350 cells/μl compared with waiting until the CD4 cell count drops below 200 cells/μl. An interim analysis of the Comprehensive International Program of Research on AIDS (CIPRA)-Haiti trial [19,20] found that initiating ART at CD4 cell counts below 200 cells/μl was associated with a four-fold increased risk of mortality and a two-fold increased risk of incident tuberculosis (TB) compared with starting at a CD4 cell count between 200 and 350 cells/μl.

The recent changes in WHO guidelines have yet to be adopted globally [21]. In order to support decision-making around when to initiate ART, we assessed the association between treatment outcomes and starting ART at higher CD4 cell counts using data collected as part of the Comprehensive International Program of Research on AIDS-South Africa (CIPRA-SA) randomized trial comparing nurse-monitored antiretroviral treatment with doctor-monitored treatment in South Africa [22].

Methods

Study design

The data for this study were collected as part of the CIPRA-SA trial, an unblinded, prospective, randomized controlled trial comparing nurse vs. doctor-monitored HIV care and demonstrated equivalence of the two monitoring strategies for treatment failure over 2 years [hazard ratio 1.09; 95% confidence interval (CI), 0.89–1.33] [22]. The study enrolled 812 HIV-positive ART-naive patients of at least 16 years of age with a CD4 cell count of 350 cells/μl or less or prior AIDS-defining illness [Centers for Disease Control and Prevention (CDC) category B/C] at one of two sites in South Africa (Soweto, Johannesburg and Masiphumelele, Cape Town). All patients were managed under South African National Guidelines for HIV treatment and were given standard ART regimens consisting of lamivudine given with either zidovudine or stavudine and either efavirenz or nevirapine [5]. A protease inhibitor-based regimen was used in a limited number of cases ($N = 62$) for women of childbearing potential with a CD4 cell count above 350 cells/μl. Patients were randomized 1 : 1 to have their HIV care monitored by either a nurse or a clinical officer and were followed for a minimum of 96 weeks. Details of the CIPRA-SA trial can be found elsewhere [22].

Patient follow-up and data collection

Patients were seen at baseline and then returned for follow-up visits at weeks 0, 2, 4, 9, 12 and then every 12 weeks. At each visit, patients had a clinical examination, symptom screening for TB and a blood draw for laboratory testing, including CD4 cell count, viral load, hematology and biochemistry.

Although current guidelines in South Africa allow for initiation of ART for patients with CD4 cell counts of 200 cells/μl or less or WHO Stage IV condition [5], and

because the CIPRA-SA study enrolled patients with a CD4 cell count of 350 cells/μl or less, we conducted a prospective cohort study assessing differences in treatment failure among those initiating ART at a CD4 cell count above 200 cells/μl and those initiating at 200 cells/μl or less.

Definition of study variables

The primary exposure was CD4 cell count above 200 vs. 200 cells/μl or less at ART initiation. CD4 cell count was measured at randomization in the main CIPRA-SA study and was assessed using $CD4^+$ flow cytometry (Flow-Count Fluorospheres; Beckman Coulter-Immunotech, Marseille, France).

We assessed the relation between starting CD4 cell count and three indicators of program failure: treatment failure (an indicator of death or failure to achieve or maintain viral suppression), incident TB and program failures (indicated by patients who leave care). Virologic failure was defined as either failure to reach a 1.5 $\log_{10}$ drop in viral load by 12 weeks on treatment or two consecutive viral loads of more than 1000 copies/ml within 1 month of each other after 24 weeks on treatment. We defined loss to follow-up (LTFU) as missing three or more consecutive study visits (in the main study shown as LTFU and defaulting clinic schedule); we did not include patients who voluntarily withdrew from the study, as these patients could remain in care just not on the study protocol.

To determine whether there were any association between toxicity-related outcomes and initiating treatment at higher CD4 cell counts, we examined the relation between initiating CD4 cell count and treatment-related toxicities. Details of the toxicities that occurred are given elsewhere [22] but included any toxicity which required discontinuation of the study regimen, with a resulting treatment interruption of more than 6 weeks.

For all analyses, person-time accrued from initiation of treatment to the date of the earliest of experiencing a treatment outcome (defined above), completion of 48 months of treatment, becoming LTFU (except in analyses in which LTFU was the outcome) or date of closing the dataset (20 January 2009).

All patients in the CIPRA-SA trial signed informed consent forms. The CIPRA-SA trial was approved by the Institutional Review Boards (IRB) of the University of the Witwatersrand and the University of Cape Town. The Boston University IRB gave approval for analysis of the data in a de-identified manner.

Statistical methods

We compared differences between study groups by stratifying our data by baseline CD4 cell count group. We looked for crude associations between baseline predictors and treatment outcomes and compared groups using relative risks (RRs) and 95% CIs. We explored the relation between initiating CD4 cell count and treatment failure by describing the rate of treatment failure using crude Kaplan–Meier curves. We used Cox proportional hazards regression to model the relation between CD4 cell count at ART initiation and treatment failure. All models were adjusted for age, sex (stratified at baseline into men, pregnant women and nonpregnant women), study site and randomization group. We did not include postbaseline measures, as these may be influenced by baseline CD4 cell count. We looked for a dose response between higher CD4 cell count and treatment outcomes by fitting a model with finer categorizations of baseline CD4 cell count. Finally, we looked for interactions between CD4 cell count and other markers of immunosuppression at baseline (e.g. viral load, WHO stage and BMI).

Results

Cohort description

Baseline characteristics of the 812 patients enrolled in the CIPRA-SA cohort stratified by baseline CD4 cell counts group are shown in Table 1. Patients were followed for a median of 27.5 months (interquartile range 13.8–33.1), with no differences by study group. As expected based on randomization, roughly half of those in each CD4 cell count group had nurse-monitored and half had doctor-monitored ART care. Median age was 32 years and more than two of three were women with no differences by CD4 cell count group. Prior ART use (typically for prevention of mother-to-child transmission) was balanced between CD4 cell count groups. Those in the low CD4 cell counts group were more likely to have nevirapine in their baseline regimen than efavirenz, whereas those in the higher CD4 cell counts group were more likely to have lopinavir–ritonavir.

Five hundred eighteen patients (64%) fell into the low CD4 cell counts group (≤200 cells/μl), whereas the remaining 294 patients (36%) were in the high CD4 cell count group (>200 cells/μl). Those in the low CD4 cell count group were more immunosuppressed at baseline as indicated by viral loads of at least 100 000 copies/ml (RR, 1.67; 95%CI, 1.43–1.95), WHO stage IV (RR, 2.32; 95% CI, 1.62–3.33) or CDC C (RR, 1.42; 95% CI, 1.16–1.73). Few patients had extremely low or high CD4 cell count; only 16% (85/518) of those in the low CD4 cell count group had a CD4 cell count below 50 cells/μl and only 16% (46/294) of those in the high CD4 cell counts group had a CD4 cell counts above 350 cells/μl.

Death and virologic failure

Treatment outcomes by CD4 cell count group are shown in Table 2. There were 21 deaths in the study (2.6%);

Table 1. Differences in baseline characteristics by baseline CD4 cell count group among 812 patients enrolled in the Comprehensive International Program of Research on AIDS-South Africa trial at two sites in South Africa.

Variable	Baseline CD4 cell count (cells/μl)		Relative risk (95% CI)
	≤200 (*N* = 518)	>200 (*N* = 294)	
Follow-up [months, median (IQR)]	27.5 (13.4–33.1)	27.5 (13.8–33.0)	
Site			
Johannesburg	299 (57.7%)	150 (51.0%)	Reference
Cape Town	219 (42.3%)	144 (49.0%)	0.86 (0.74–1.01)
Study group			
Primary healthcare nurse	260 (50.2%)	144 (49.0%)	Reference
Medical officer	258 (49.8%)	150 (51.0%)	0.98 (0.85–1.12)
Sex			
Male	155 (29.9%)	84 (28.6%)	Reference
Female	363 (70.1%)	210 (71.4%)	0.98 (0.90–1.08)
Age at baseline, median (IQR)	32 (28.1–37.3)	32.5 (27.8–36.7)	
Race			
Black/African	515 (99.4%)	289 (98.3%)	Reference
Mixed race	3 (0.6%)	5 (1.7%)	0.34 (0.08–1.41)
Baseline ART regimen			
d4T–3TC–EFV	370 (71.4%)	227 (77.2%)	Reference
d4T–3TC–NVP	119 (23.0%)	34 (11.6%)	1.87 (1.32–2.65)
d4T–3TC–LPVr	25 (4.8%)	30 (10.2%)	0.54 (0.33–0.90)
d4T–3TC–NLF	4 (0.8%)	3 (1.0%)	0.82 (0.19–3.63)
Prior ART exposure			
None	390 (75.3%)	219 (74.5%)	Reference
sdNVP	113 (21.8%)	54 (18.4%)	1.14 (0.85–1.52)
ZDV mono	2 (0.4%)	4 (1.4%)	0.28 (0.05–1.54)
NVP/ZDV	13 (2.5%)	16 (5.4%)	0.47 (0.23–0.97)
ART	0 (0%)	1 (0.3%)	
WHO stage at baseline			
1	71 (13.8%)	80 (28.0%)	Reference
2	154 (29.8%)	96 (33.6%)	1.25 (1.07–1.47)
3	197 (38.2%)	84 (29.4%)	1.44 (1.22–1.69)
4	94 (18.2%)	26 (9.1%)	2.32 (1.62–3.33)
CDC category			
A	177 (34.2%)	124 (42.2%)	Reference
B	135 (26.1%)	94 (32.0%)	1.00 (0.82–1.22)
C	206 (39.8%)	76 (25.9%)	1.42 (1.16–1.73)
CD4 cell count at baseline, median (IQR)	123 (77–159)	254.5 (224–308)	
Baseline hemoglobin, median (IQR)	11.7 (10.3–13.1)	12.4 (11.4–13.5)	
BMI at baseline, median (IQR)	23.1 (20.5–26.6)	24.1 (21.3–28.4)	
Baseline viral load			
<100 000	174 (33.6%)	177 (60.2%)	Reference
≥100 000	344 (66.4%)	117 (39.8%)	1.67 (1.43–1.95)
Viral load at baseline (×1000), median (IQR)	201.5 (64.8–525.0)	66.9 (20.9–188.0)	

3TC, lamivudine; ART, antiretroviral therapy; CI, confidence interval; CIPRA-SA, Comprehensive International Program of Research on AIDS-South Africa; d4T, stavudine; EFV, efavirenz; IQR, interquartile range; LPVr, lopinavir–ritonavir; NLF, nelfinavir; NVP, nevirapine; sdNVP, single-dose nevirapine; ZDV, zidovudine.

however, in crude analyses, those with a baseline CD4 cell count of 200 cells/μl or less had a five-fold increased risk vs. those above 200 cells/μl (RR, 5.4; 95% CI, 1.3–23.0). Virologic failure occurred in 10% of the cohort, with the majority (84%) of these virologic failures based on two consecutive viral loads of above 1000 copies/ml and not based on failure to achieve a 1.5 $\log_{10}$ drop in viral load from baseline by 12 weeks (16%, 13/83). Those with a CD4 cell count of 200 cells/μl or less at baseline had nearly twice the crude risk of virologic failure as those above 200 cells/μl (RR, 1.79; 95% CI 1.10–2.90). The Kaplan–Meier curve of death or virologic failure presented in Fig. 1(a) shows that the difference between the two groups in death and virologic failure emerges mainly between 6 and 24 months on treatment.

Table 3 shows three different crude and adjusted models of the relation between baseline CD4 cell counts and virologic failure or death. We present three separate models that are identical except that they use different categorizations of CD4 cell count (models 1 and 2) or include an interaction between baseline WHO stage and CD4 cell count (model 3). Model 1, which uses the CD4 categories used previously (≤200 vs. >200 cells/μl), shows that after adjusting for age, sex and pregnancy, site, treatment arm and other indicators of immunosuppression, those with a baseline CD4 cell count of 200 cells/μl or less had twice the risk of virologic failure or death (hazard ratio, 1.94; 95% CI, 1.14–3.30). Having a baseline WHO stage IV (hazard ratio, 1.98; 95% CI, 1.18–3.33), a viral load above 100 000 vs. below

Table 2. Reasons for antiretroviral therapy treatment failure stratified by baseline CD4 cell count group among 812 patients enrolled in the Comprehensive International Program of Research on AIDS-South Africa trial at two sites in South Africa.

	Baseline CD4 cell count group			
	≤200, $N=518$	>200, $N=294$	Total, $N=812$	Relative risk (95% CI)
Outcome				
Death	19/518 (3.7%)	2/294 (0.7%)	21 (2.6%)	5.39 (1.26–22.0)
Virologic failure	63/518 (12.2%)	20/294 (6.8%)	83 (10.2%)	1.79 (1.10–2.90)
1.5 log drop[a]	8/518 (1.5%)	5/294 (1.7%)	13 (1.6%)	0.91 (0.30–2.75)
2 Viral load >1000[b]	55/518 (10.6%)	15/294 (5.1%)	70 (8.6%)	2.08 (1.20–3.62)
Incident tuberculosis	41/518 (8.0%)	9/294 (3.1%)	50 (6.2%)	2.59 (1.27–5.24)
Other study outcomes				
Toxicity failure	81/518 (15.6%)	53/294 (18.0%)	134 (16.5%)	0.87 (0.63–1.19)
LTFU	52/518 (10.0%)	42/294 (14.3%)	94 (11.6%)	0.70 (0.48–1.03)
Default clinic schedule	40/518 (7.7%)	30/294 (10.2%)	70 (8.6%)	0.76 (0.48–1.19)
LTFU	12/518 (2.3%)	12/294 (4.1%)	24 (3%)	0.57 (0.26–1.25)

CI, confidence interval; CIPRA-SA, Comprehensive International Program of Research on AIDS-South Africa; LTFU, loss to follow-up.
[a]Failure to achieve a 1.5 $\log_{10}$ drop in viral load from baseline at 12 weeks on treatment.
[b]Two consecutive viral loads >1000 copies/ml within 1 month of each other after 24 weeks on treatment.

10 000 copies/ml (hazard ratio, 2.05; 95% CI, 0.71–5.89) and having a nelfinavir-based regimen (hazard ratio, 4.27; 95% CI, 1.17–15.6) were also predictive of virologic failure or death independent of CD4 cell counts.

In Model 2, we looked for a CD4 cell count dose response between decreasing baseline CD4 cell count and risk of death or virologic failure by stratifying baseline CD4 cell count into finer categories (0–99, 100–199, 200–299, and ≥300 cells/μl). After adjustment, we found those with a CD4 cell count between 100 and 199 cells/μl and those with a CD4 cell count below 100 cells/μl had a roughly three-fold increased risk of death or virologic failure as those with at least 300 cells/μl (CD4 cell count <100 vs. >300 cells/μl; hazard ratio, 3.08; 95% CI, 0.92–10.4 and CD4 cell count 100–199 vs. >300 cells/μl, hazard ratio, 3.23; 95% CI, 0.99–10.6 cells/μl), whereas those with a CD4 cell count of

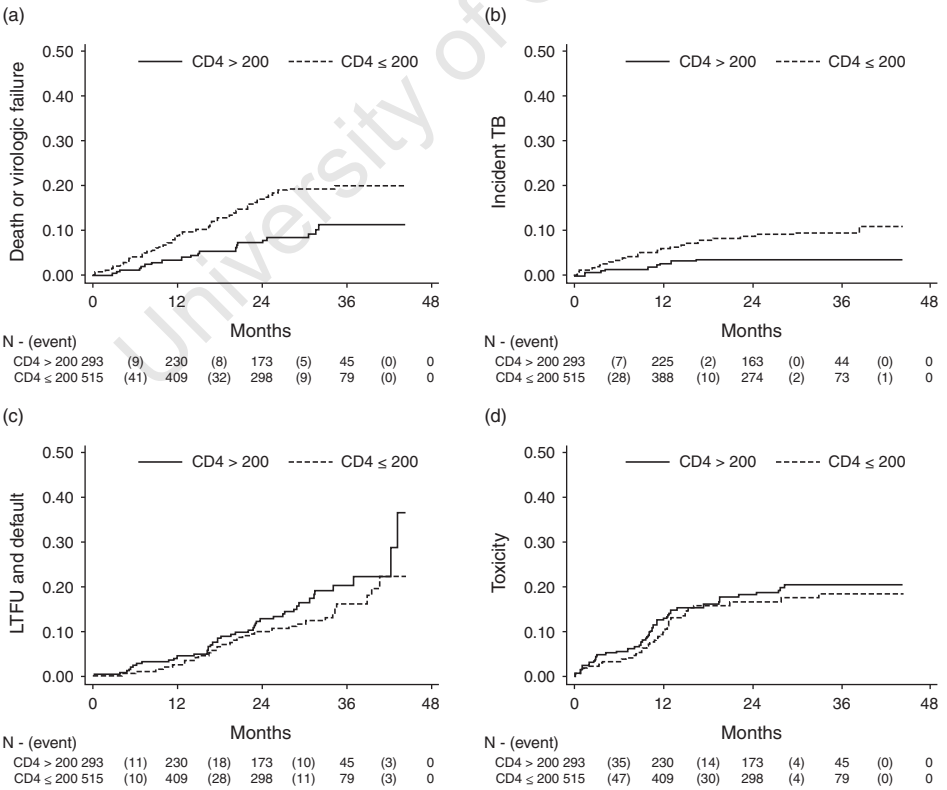

Fig. 1. Kaplan–Meier analysis. Time to (a) death or virologic failure, (b) tuberculosis, (c) LTFU and (d) toxicity by baseline CD4 cell count group among 812 patients enrolled in the CIPRA-SA trial at two sites in South Africa. Note that *y*-axis goes from 0 to 0.5. CIPRA-SA, Comprehensive International Program of Research on AIDS-South Africa; LTFU, loss to follow-up; TB, tuberculosis.

Table 3. Crude and adjusted hazard ratios of death or virologic failure among 812 patients enrolled in the Comprehensive International Program of Research on AIDS-South Africa trial at two sites in South Africa.

	Model 1[a]		Model 2[a]		Model 3[a]	
Predictor of death or viral load failure	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Age at baseline (continuous)	1.00 (0.97–1.03)	1.01 (0.98–1.04)	1.00 (0.97–1.03)	1.01 (0.98–1.04)	1.00 (0.97–1.03)	1.01 (0.98–1.04)
BMI at baseline (continuous)	0.99 (0.95–1.03)	1.00 (0.96–1.04)	0.99 (0.95–1.03)	1.00 (0.96–1.04)	0.99 (0.95–1.03)	1.00 (0.96–1.04)
Pregnant vs. not pregnant women	1.60 (0.99–2.58)	1.53 (0.80–2.91)	1.60 (0.99–2.58)	1.51 (0.79–2.87)	1.60 (0.99–2.58)	1.52 (0.80–2.89)
Men vs. not pregnant women	0.94 (0.62–1.43)	1.03 (0.62–1.71)	0.94 (0.62–1.43)	1.02 (0.62–1.69)	0.94 (0.62–1.43)	1.02 (0.62–1.69)
Johannesburg vs. Cape Town	1.22 (0.82–1.81)	1.23 (0.79–1.92)	1.22 (0.82–1.81)	1.20 (0.77–1.88)	1.22 (0.82–1.81)	1.22 (0.78–1.90)
Nurse vs. doctor	1.10 (0.75–1.62)	1.13 (0.75–1.69)	1.10 (0.75–1.62)	1.14 (0.76–1.71)	1.10 (0.75–1.62)	1.10 (0.74–1.64)
Regimen with LPVr vs. NNRTI[a]	1.41 (0.71–2.79)	1.57 (0.69–3.58)	1.41 (0.71–2.79)	1.54 (0.67–3.52)	1.41 (0.71–2.79)	1.51 (0.66–3.44)
Regimen with NLF vs. NNRTI[a]	3.92 (1.24–12.4)	4.27 (1.17–15.6)	3.92 (1.24–12.37)	4.20 (1.14–15.4)	3.92 (1.24–12.4)	3.56 (0.98–13.0)
Viral load >100 000 vs. <10 000	1.77 (0.69–4.55)	2.41 (0.86–6.78)	1.77 (0.69–4.55)	2.40 (0.85–6.76)	1.77 (0.69–4.55)	1.89 (0.74–4.82)
Viral load 10 000–100 000 vs. <10 000	2.43 (0.98–6.03)	2.05 (0.71–5.89)	2.43 (0.98–6.03)	2.05 (0.71–5.89)	2.43 (0.98–6.03)	1.58 (0.61–4.12)
CD4 cell count ≤200 vs. >200, cells/μl	2.11 (1.32–3.38)	1.94 (1.14–3.30)				
CD4 cell count 0–99 vs. ≥300, cells/μl			3.29 (1.16–9.34)	3.08 (0.92–10.4)		
CD4 cell count 100–199 vs. ≥300, cells/μl			3.17 (1.15–8.76)	3.23 (0.99–10.6)		
CD4 cell count 200–299 vs. ≥300, cells/μl			1.72 (0.58–5.08)	1.86 (0.54–6.45)		
WHO Stage IV vs. III/II/I	1.81 (1.13–2.90)	1.98 (1.18–3.33)	1.81 (1.13–2.90)	2.00 (1.19–3.37)		
WHO IV, CD4 cell count >200 vs. no WHO IV, CD4 cell count >200					2.64 (0.89–7.80)	2.87 (0.93–8.83)
No WHO IV, CD4 cell count ≤200 vs. no WHO IV, CD4 cell count >200					2.24 (1.33–3.78)	2.14 (1.20–3.81)
WHO IV, CD4 cell count ≤200 vs. no WHO IV, CD4 cell count >200					3.36 (1.75–6.46)	3.91 (1.88–8.14)

CI, confidence interval; CIPRA-SA, Comprehensive International Program of Research on AIDS-South Africa; HR, hazard ratio; LPVr, lopinavir–ritonavir; NLF, nelifinavir; NNRTI, nonnucleoside reverse transcriptase inhibitor.

[a]Crude models are adjusted for each predictor by itself, whereas adjusted models are adjusted for all other variables in the model. All adjusted models included regimen age, sex, site, randomization group, baseline viral load and BMI. Model 1 included dichotomous predictors for baseline CD4 cell count (≤200 vs. >200 cells/μl) and WHO stage (IV vs. III/II/I) with no interaction terms. Model 2 included a dichotomous predictor for baseline WHO stage (IV vs. III/II/I) and four categories for baseline CD4 cell count (0–99, 100–199, 200–299 vs. >300 cells/μl) with no interaction terms. Model 3 included dichotomous predictors for CD4 cell count (≤200 vs. >200 cells/μl) and WHO stage (IV vs. III/II/I) as well as an interaction term between baseline CD4 cell count and WHO stage.

Table 4. Crude and adjusted hazard ratios of incident tuberculosis among 812 patients enrolled in the Comprehensive International Program of Research on AIDS-South Africa trial at two sites in South Africa.

	Model 1[a]		Model 2[a]	
Predictor of death or viral load failure	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Age at baseline (continuous)	1.02 (0.98–1.06)	1.02 (0.98–1.06)	1.02 (0.98–1.06)	1.02 (0.98–1.06)
BMI at baseline (continuous)	0.90 (0.84–0.97)	0.91 (0.84–0.99)	0.90 (0.84–0.97)	0.91 (0.84–0.99)
Women	0.71 (0.40–1.26)	1.30 (0.66–2.55)	0.71 (0.40–1.26)	1.29 (0.65–2.54)
Johannesburg vs. Cape Town	0.58 (0.33–1.02)	0.60 (0.32–1.12)	0.58 (0.33–1.02)	0.69 (0.36–1.32)
Nurse vs. doctor	0.92 (0.53–1.60)	1.06 (0.59–1.89)	0.92 (0.53–1.60)	1.03 (0.58–1.84)
Regimen with a protease inhibitor[a]	0.54 (0.13–2.22)	0.93 (0.21–4.04)	0.54 (0.13–2.22)	0.98 (0.22–4.27)
WHO stage IV vs. III/II/I	2.09 (1.09–4.00)	1.27 (0.63–2.56)	2.09 (1.09–4.00)	1.12 (0.55–2.29)
Viral load >100 000 vs. <10 000	2.37 (0.73–7.66)	1.23 (0.36–4.22)	2.37 (0.73–7.66)	1.18 (0.34–4.07)
Viral load 10 000–100 000 vs. <10 000	0.65 (0.17–2.51)	0.53 (0.13–2.08)	0.65 (0.17–2.51)	0.50 (0.13–1.97)
CD4 cell count ≤200 vs. >200, cells/μl	2.64 (1.28–5.43)	1.90 (0.89–4.04)		
CD4 cell count 0–49 vs. ≥200, cells/μl			5.95 (2.57–13.8)	3.39 (1.35–8.51)
CD4 cell count 50–199 vs. ≥200, cells/μl			2.05 (0.96–4.36)	1.60 (0.73–3.51)

CI, confidence interval, CIPRA-SA, Comprehensive International Program of Research on AIDS-South Africa; HR, hazard ratio.
[a]Crude models are adjusted for each predictor by itself, whereas adjusted models are adjusted for all other variables in the model. All adjusted models included regimen, age, sex, site, randomization group, baseline viral load, WHO stage and BMI. Model 1 included a dichotomous predictor for baseline CD4 cell count (≤200 vs. >200 cells/μl). Model 2 included three categories for baseline CD4 cell count: 0–49, 150–199, ≥200 cells/μl).

200–299 cells/μl had twice the risk (hazard ratio, 2.00; 95% CI, 1.19–3.37). Thus, higher baseline CD4 cell count does appear to be associated with lower risk of virologic failure and death.

We also looked for interactions between the baseline CD4 cell count group and other markers of immunosuppression. Model 3 shows the results of a regression model that includes the only significant interaction we identified, that between baseline CD4 cell count (≤200 vs. >200 cells/μl) and baseline WHO Stage IV. Compared with the reference group of those with a baseline CD4 cell count above 200 cells/μl and no WHO Stage IV condition, those with either a WHO Stage IV condition alone or a CD4 cell count of 200 cells/μl or less alone had a two-to-three-fold increased risk of death or virologic failure (hazard ratio, 2.87; 95% CI, 0.93–8.83 and hazard ratio, 2.14; 95% CI, 1.20–3.81, respectively). However, those with both a WHO Stage IV condition and a CD4 cell count of 200 cells/μl or less at baseline had a four-fold increased risk of death or virologic failure vs. those with neither condition (hazard ratio, 3.91; 95% CI, 1.88–8.14).

Tuberculosis

Overall about 6% of all patients in the study developed incident TB over the course of follow-up with substantially more of it occurring among those initiated on ART at CD4 cell counts of 200 cells/μl or less than among those initiated above 200 cells/μl (8.0 vs. 3.1%; RR, 2.6; 95% CI, 1.3–5.2) (Table 2). In a Kaplan–Meier analysis (Fig. 1c), we note that the majority of the difference between the two groups emerged within the first 24 months on treatment. In Table 4, we present two separate models of incident TB that are identical except that they use different categorizations of CD4 cell count (Models 1 uses ≤200 vs. >200 cells/μl, whereas Model 2 uses <50, 50–199 and ≥200 cells/μl). After adjusting for site, treatment group, age, sex, use of a protease inhibitor-based regimen, baseline viral load, WHO stage and BMI, using proportional hazards regression, we found that those initiated on ART at CD4 cell counts of 200 cells/μl were twice as likely to develop TB compared with those initiated above 200 cells/μl (hazard ratio, 1.9; 95% CI, 0.89–4.0) (Table 4, Model 1). When we further stratified the lowest CD4 cell counts group into those above and below 50 cells/μl (Model 2), we found that those with a CD4 cell count below 50 vs. above 200 cells/μl were at strongly increased risk of incident TB (hazard ratio, 3.4; 95% CI, 1.4–8.5), whereas those with 50–200 vs. above 200 cells/μl were at lower increased risk (hazard ratio, 1.60; 95% CI, 0.73–3.5) (Table 4).

Loss to follow-up

Nearly 12% of the cohort were LTFU and LTFU was more common among those with a baseline CD4 cell count above 200 than 200 cells/μl or less (14.3 vs. 10.2%)(Table 2). After adjusting for age, sex, baseline WHO stage, viral load, use of a protease inhibitor, treatment arm and site (data not shown), we found a small decreased risk of being LTFU among those initiated on ART with lower CD4 cell counts (≤200 cells/μl) vs. those initiated at higher CD4 cell counts (>200 cells/μl) (hazard ratio, 0.79; 95% CI, 0.50–1.25). Figure 1(b) shows that the difference between the groups emerges only after 24 months, the minimum potential follow-up time for the cohort. Those with a baseline WHO stage IV condition also had an increased risk of LTFU (hazard ratio, 1.61; 95% CI 0.93–2.79) (data not shown).

Toxicities

Toxicity endpoints were experienced by about 17% of the entire cohort (Table 2). We observed small differences in rates of toxicities between CD4 count

groups slightly favoring those with baseline CD4 cell counts of 200 cells/μl or less vs. above 200 cells/μl (15.6 vs. 18.0%) (Fig. 1d). After adjusting for age, sex, baseline WHO stage, viral load, treatment regimen, treatment arm and site, women (hazard ratio, 1.95; 95% CI, 1.17–3.24), those who with a BMI above 30 vs. 18.5–30 kg/m^2 (hazard ratio, 2.17; 95% CI, 1.41–3.34) or a baseline WHO stage IV condition (hazard ratio, 1.91; 95% CI, 1.15–3.18) were at increased risk of toxicity failure, but we found little difference between CD4 cell count groups (hazard ratio, 0.78; 95% CI, 0.53–1.16) (data not shown). Use of lopinavir–ritonavir vs. efavirenz-based regimens decreased the risk of toxicity (hazard ratio, 0.40; 95% CI, 0.16–1.02). Nevirapine use was associated with increased risk of toxicity vs. efavirenz-based regimens in unadjusted analyses (hazard ratio, 1.46; 95% CI, 0.99–2.16), but the association disappeared after adjustment (hazard ratio, 1.01; 95% CI, 0.63–1.61).

Discussion

The results of analyses of the CIPRA-SA trial data show a clearly increased risk of death or virologic failure associated with initiating ART at lower CD4 cell counts. We found that those who started ART at CD4 cell counts of 200 cells/μl or less had roughly twice the risk of death or virologic failure as those initiated at CD4 cell counts above 200 cells/μl (hazard ratio, 1.94; 95% CI, 1.14–3.30) and twice the risk of developing incident TB (hazard ratio, 1.90; 95% CI, 0.89–4.04). These findings are in line with numerous observational studies [23–27] from resource-limited settings showing low baseline CD4 cell count is a major predictor of death and LTFU. Recently, an interim analysis of the CIPRA-HT001 trial in Haiti showed a nearly four-fold increased risk of death among those starting ART with a CD4 cell count of 200 vs. 200–350 cells/μl [19], very similar to our five-fold increased risk (RR, 5.4; 95% CI, 1.3–23.0) [19]. Thus, a body of evidence is beginning to emerge, showing the benefits of earlier treatment initiation in resource-limited settings. This, along with a recent analysis by Lawn *et al.* [28] showing that the longer a patient maintains a CD4 cell count of below 100 cells/μl, the higher the risk of death suggests that starting patients at higher CD4 cell counts may allow them to maintain their CD4 cell counts above the point at which they are at increased risk of death.

Our findings are also consistent with data from resource-rich environments. Observational data have shown that higher CD4 cell counts are associated with lower risk of death [7,9]. More recently, Kitahata *et al.* [13] have shown that patients initiating ART at CD4 cell counts above 500 cells/μl had substantially reduced risk of mortality vs. those below 500 cells/μl. Although our data cannot be used to comment on CD4 cell counts above 350 cells/μl, our finding of decreased mortality and virologic failure risk associated with having a starting CD4 cell count above 300 cells/μl is suggestive of a dose response with increasing baseline CD4 cell count associated with better outcomes.

Although we found a substantial association between earlier ART initiation and better treatment outcomes we also found a slightly increased risk of being LTFU among those with higher baseline CD4 cell counts, which could potentially offset some of the benefits of initiating treatment earlier. However, we urge caution in interpreting these results. Under ideal conditions, assessing the effectiveness of initiating ART at higher CD4 cell counts would come from randomizing patients to either immediate initiation of ART when the CD4 cell count falls below 350 cells/μl or follow patients and delay ART until the CD4 cell count falls below 200 cells/μl [14,29]. In both arms, patients would be followed from the time of their first CD4 cell count below 350 cells/μl. In our study, patients were initiated onto ART at enrollment as long as their CD4 cell count was below 350 cells/μl, so we do not have any follow-up time to approximate what happens to patients in the time their CD4 cell count is between 200 and 350 cells/μl; however, we anticipate some deaths and LTFU occur in this time. Although methods exist to adjust for this lead-time bias [30], they require pre-ART data, which we did not have.

The current analysis has several strengths. The data were from a large prospective randomized trial with excellent follow-up data at standardized intervals, which allowed the assessment of the impact of starting treatment at higher CD4 cell counts. Although the data were from a randomized trial of another intervention, because the trial showed no differences between randomization groups (i.e. nurse vs. doctor-monitored care) and because adjustment for randomization group had no impact on our current results, there is little evidence that the primary intervention had any impact on our findings.

Still, the current analysis should be considered in light of several limitations. First, as noted above, we did not have the ideal comparison group to assess death and virologic failure (i.e. a group followed from a CD4 cell count of 350 cells/μl until 200 cells/μl and then initiated on ART). Thus, any deaths occurring between 350 and 200 cells/μl would not be included in our analysis. As we are missing deaths in the high CD4 cell count group, this analysis may underestimate the treatment benefits of starting at higher CD4 cell counts. Thus, our estimates cannot be considered the true causal effect of starting treatment at higher CD4 cell counts. Second, as the data came from a randomized trial with conservative definitions of toxicity, many patients who were treatment failures for toxicity might have otherwise continued on treatment under usual practice conditions. This could

have biased our toxicity results toward the null and prevented us from observing a true difference between the groups if one existed. Third, in our analysis of LTFU, we were not able to determine the final outcomes of patients lost and, therefore, we cannot say whether patients left care because they were feeling well nor could we determine how many of them have since died. Finally, as the data were from a trial, the study population may have been healthier than the general clinic population.

Conclusion

We found that patients initiated on standard first-line South African ART regimens were at increased risk of death and virologic failure if initiated at CD4 cell counts below 200 cells/μl compared with those initiated above 200 cells/μl. This is consistent with findings from developed areas that have shown that the benefits of starting at earlier CD4 cell counts outweigh the risks of toxicity and long-term adherence. Although the cost implications are unknown, national and international guidelines on the topic of when to initiate ART should consider our findings when deciding on whether to increase initiating CD4 cell count thresholds. If thresholds are increased, then substantial efforts will need to be made to move patients into care earlier in their disease progression in order to obtain the maximum benefit from ART.

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M.F. performed the primary statistical analyses and drafted the manuscript. I.S., F.C. J.Z., C.O. R.I. M.R. M.D. C.v.d.H. J.M. and R.W. contributed to the design of the study and data interpretation and revising the article. All authors approved the final manuscript.

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Optimum time to start antiretroviral therapy during HIV-associated opportunistic infections

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Purpose of review

We review recently published literature concerning the optimum time to start antiretroviral therapy (ART) in patients with HIV-associated opportunistic infections.

Recent findings

In addition to data from observational studies, results from six randomized controlled clinical trials were available by July 2010. The collective findings of these trials were that patients with CD4 cell counts less than 200 cells/μl who start ART within the first 2 weeks of treatment for opportunistic infections including *Pneumocystis jirovecii* pneumonia, serious bacterial infections or pulmonary tuberculosis have lower mortality when compared to patients starting ART at later time-points. Moreover, patients with pulmonary tuberculosis and CD4 counts of 200–500 cells/μl who started ART during tuberculosis (TB) treatment had improved survival compared to those who deferred ART until after the end of treatment. In contrast, in two separate studies, immediate ART conferred no survival benefit in patients with TB meningitis and was associated with substantially higher mortality risk in patients with cryptococcal meningitis.

Summary

Initiation of ART during the first 2 weeks of treatment for serious opportunistic infections has been shown to be associated with improved survival with the exception of patients with tuberculous meningitis and cryptococcal meningitis. Further clinical trials are ongoing.

Keywords

antiretroviral, HIV, opportunistic infection, timing, when to start

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Introduction

Remarkable progress has been made over the past 15 years in the treatment of HIV infection such that average additional life expectancy of young adults initiating antiretroviral therapy (ART) in high-income countries is estimated to be in the region of 50 years [1]. However, mortality remains unacceptably high among those patients who present to ART services with advanced immunodeficiency and serious opportunistic infections and especially in resource-limited settings [2–4]. It is as yet unclear when patients should start ART during the treatment for their opportunistic infections to minimize this mortality risk. In this paper, we discuss the rationale for either early or deferred initiation of ART. We review insights provided by observational cohort studies and the key evidence that is now emerging from randomized controlled trials.

Rationale for early or late initiation of antiretroviral therapy

The rationale for either early or deferred initiation of ART is defined by a range of potential factors (Table 1). Early ART halts progressive immunodeficiency and rapid immune recovery may reduce the risks of further opportunistic infections and mortality. It may also promote more rapid immune clearance of the opportunistic infections and reduce the risk of relapse [5]. Moreover, for some infections such as cryptosporidiosis, microsporidiosis and progressive multifocal leucoencephalopathy, ART represents the most important component of treatment and should therefore not be delayed at all.

For other serious opportunistic infections such as tuberculosis (TB) and cryptococcal meningitis, however, there are several adverse consequences associated with early initiation of ART, which may favour delaying treatment (Table 1). The high pill burden with overlapping regimens may reduce treatment tolerability and undermine adherence. In addition, there may be pharmacokinetic drug–drug interactions and cotoxicities. An important example of this is the concurrent use of rifampicin-containing TB treatment and ART regimens containing either non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors [6,7]. Moreover, in patients who develop suspected adverse reactions to

Table 1 Potential advantages and disadvantages of starting antiretroviral therapy early in the course of treatment for serious opportunistic infections

Potential advantages of early initiation of ART	Potential disadvantages of early initiation of ART (overlapping treatment)
Prevent progressive immunodeficiency	High pill burden
More rapid immune recovery	Cotoxicity
More rapid resolution of OI	Pharmacokinetic drug interactions
Rapid reduction in mortality risk	Immune reconstitution disease
Prevention of further OIs and other morbidity	More difficult to identify drug causing toxicity

ART, antiretroviral therapy; OI, opportunistic infection.

drugs, identifying the cause is more complex when multiple drugs are started simultaneously.

Paradoxical immune reconstitution disease (IRD; also known as immune reconstitution inflammatory syndrome or IRIS) and its consequences is one of the most important considerations and is frequently cited as a key reason for treatment deferral. This complication arises as an inflammatory response to residual microbial antigen during rapid immune recovery [8,9]. The risk of IRD is therefore higher the earlier ART is started [10,11]. A particular concern is that IRD associated with opportunistic infections involving the central nervous system (CNS) may be associated with higher mortality risk than that associated with IRD occurring outside the CNS [12].

Thus, the optimal timing of ART depends on a number of important competing risks. For many years this has been associated with considerable clinical uncertainty and yet data from many observational studies and a number of controlled clinical trials are now available.

Observational studies

Studies from South Africa have highlighted the high mortality risk of adults and children waiting to start ART [13–15]; even short delays of a few weeks may be associated with substantial mortality risk. Mortality risk before and during ART are higher for patients initiating treatment in resource-limited settings [16]. In sub-Saharan Africa, for example, between 8 and 26% of patients die in the first year of ART [4]. A high proportion of these deaths occur in the first 3 months of ART and mortality risk in this period is several-fold higher than that of patients treated in high-income settings even after adjustment for the degree of immunodeficiency and other baseline patient characteristics [3]. Deferral of ART may therefore be associated with greater risks for patients treated in sub-Saharan Africa and the optimum timing for ART initiation during opportunistic infections may differ between settings [17].

In patients with HIV-associated TB, there was concern that poor tolerability of concurrent treatment regimens and reduced plasma concentrations of NNRTIs and protease inhibitors due to induction of hepatic metabolism by rifampicin would undermine virological outcomes [6,7]. However, excellent virological outcomes have subsequently been reported, regardless of whether patients were treated using a simplified public health approach in resource-limited settings or with highly individualized treatment in high-income settings [18–20]. Similarly, despite fears of cotoxicity from concurrent use of ART and TB treatment, treatment-limiting toxicity is not common in cohorts receiving NNRTI-based ART [21–23].

Perhaps the greatest concern among those favouring deferred initiation of ART in patients with TB was that of IRD [8]. A small proportion of patients with TB IRD die, and yet this risk has to be understood from the perspective that those who are most likely to develop this complication are the very patients who have the highest pre-existing mortality risk [8]. A recent systematic review and meta-analysis reported a pooled incidence estimate of TB IRD of 15.7% [95% confidence interval (CI) 9.7–24.5] among patients receiving overlapping TB treatment and ART [24•]. Of those developing this complication, 3.2% (0.7–9.2) died [24•], indicating that approximately 1 in 200 (0.5%) patients receiving concurrent TB treatment and ART die from (or with) this complication.

The optimal timing of ART initiation may depend on the opportunistic infection and its anatomical location. IRD involving the CNS, for example, is generally more severe and associated with higher mortality risk [12]. A meta-analysis of studies of patients with cryptococcal meningitis starting ART found a pooled incidence of IRD of 19.5% (95% CI 6.7–44.8) and of these patients 20.8% (5.0–52.7) died [24•]. Thus, approximately 1 in 25 patients starting ART during treatment for cryptococcal meningitis died of immune reconstitution disease. In a South African study of 23 patients with paradoxical TB IRD involving the CNS, 87% required hospital admission, 91% received corticosteroids and 13% died during the 6-month follow-up period [25•]. Thus, early ART initiation in patients with CNS opportunistic infections may be associated with adverse overall outcomes.

Researchers have attempted to delineate the optimal timing of ART from observational cohorts of patients with TB. A retrospective analysis of 1003 Thai patients

demonstrated that patients with HIV-associated TB who delayed ART for at least 6 months after TB diagnosis had a higher mortality rate than those who initiated ART less than 6 months after TB diagnosis (hazard ratio 2.65, 95% CI 1.15–6.10) [26]. Velasco and colleagues [27•] in Spain studied 313 adult patients with HIV-associated TB who received overlapping therapy. Groups of patients who started ART either within the first 2 months of TB treatment or after completing at least 3 months of TB treatment had similar median baseline CD4 cell counts but the adjusted hazards of death among those starting ART within 2 months of TB treatment was 0.37 (95% CI 0.17–0.66). A major weakness in this analysis, however, is that it did not account for patients who died before starting ART nor for biases inherent in ART allocation.

Observational data have also been derived from an analysis of a large paediatric cohort of children with HIV-associated TB ($n = 573$) in South Africa [28•]. Mortality risk was calculated stratified according to the timing of ART during TB treatment. The authors report a statistically nonsignificant trend in results; delay in ART for more than 60 days compared to less than 60 days was associated with hazard of death of 1.32 (95% CI 0.55–3.16) and a hazard of viral suppression of 0.84 (95% CI 0.61–1.15). Such analyses remain fundamentally flawed even after adjustment for baseline immunodeficiency and other characteristics. The timing of ART initiation is a clinically based decision and so sicker patients with more advanced immunosuppression (and higher mortality risk) are much more likely to start ART early in the course of TB treatment. How this decision is made cannot be fully adjusted for in observational data, which therefore remain confounded by indication.

Randomized controlled trials

Observational studies have proven useful in defining the competing risks inherent in the clinical decision making, informing early versions of treatment guidelines and shaping the subsequent design of controlled clinical trials. Well conducted randomized clinical trials are, however, needed to provide definitive data to underpin public health policy. By July 2010, data were available from five randomized controlled trials (RCTs) in which mortality was included in the primary outcome [29••,30,31••,32••,33]. These studies enrolled patients with a range of opportunistic infections in different geographical settings (Table 2) and below we discuss each of these in turn.

A sixth study was a pilot study in Tanzania that assessed the impact of delayed versus early initiation of a triple-nucleoside regimen on adverse events including IRD in patients ($n = 70$) being treated for TB [34•]. Both early and late ART initiation was well tolerated and no IRD events were diagnosed.

Acute opportunistic infections excluding tuberculosis

The AIDS Clinical Trials Group (ACTG) study A5164 was the first randomized controlled trial to be reported and enrolled most of its participants in the USA (Table 2) [29••]. Patients had very advanced immunodeficiency (median CD4 cell count, 29 cells/μl) and a range of opportunistic infections, excluding TB. A majority (63%) had *Pneumocystis jirovecii* pneumonia (excluding severe disease), with the other most frequent opportunistic infections being bacterial infections, cryptococcosis and toxoplasmosis (Table 2). Patients were randomized to start ART within the first 14 days of opportunistic infection treatment (median 12 days) or to start ART after completion of opportunistic infection treatment (median 45 days; IQR 41–55) and were followed up for 48 weeks.

The primary end-point was a composite three-level ordered categorical variable that included death, progression to AIDS and virological response. A trend favouring earlier treatment, however, was not statistically significant. Because virological responses at 48 weeks in the two groups were equivalent, inclusion of virological suppression within the composite primary end-point effectively 'diluted' the observed differences in the important clinical outcomes. The simpler (and perhaps more appropriate) secondary end-point of death or progression to AIDS was strongly associated with the timing of ART (Fig. 1). The early arm had fewer patients with progression to AIDS or death compared to the late arm (14.2 versus 24.1%; odds ratio = 0.51, 95% CI 0.27–0.94). Early ART was also strongly associated with a shorter time to achieving a CD4 cell count greater than 50 cells/μl (4.0 versus 8.6 weeks, $P < 0.001$) and no increase in adverse events or immune reconstitution disease.

These data therefore provided important evidence to support early initiation of ART in patients presenting with acute AIDS-related opportunistic infections or severe bacterial infections with the exclusion of TB. There were, however, an insufficient number of patients with cryptococcal meningitis to inform management of this condition although there was a very strong trend towards lower progression to AIDS or death in those receiving early treatment.

Pulmonary tuberculosis in South Africa

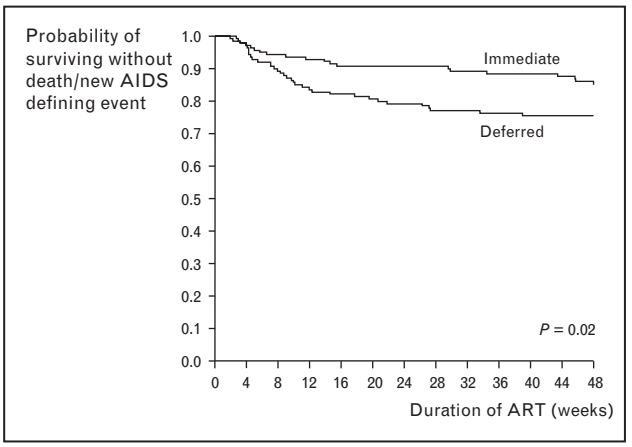
In an open-label, randomized controlled trial in Durban, South Africa (the 'SAPIT' trial), HIV-infected patients with sputum smear-positive pulmonary TB were assigned to start ART within the first 4 weeks of the intensive phase of TB treatment (early integrated arm), within the first 4 weeks of the continuation phase (late integrated arm) or within 4 weeks of completing TB

Table 2 Summary of data available from randomized controlled trials to date

Study	Study name/acronym and registration	Country	*N*	Opportunistic infection(s)	Comparison	Median (IQR) CD4 counts (cells/μl)	Outcome
Zolopa *et al.* [29••]	ACTG A5164; NCT00055120	USA, Puerto Rico, South Africa (multicentre)	282	Acute AIDS-related OIs or severe bacterial infections excluding TB (63% PCP, 12% bacterial infections, 12% cryptococcal disease, 5% toxoplasmosis, 8% other)	Early arm: started ART within 14 days of starting treatment for OI (median = 12 days) Deferred ART: started ART after OI treatment completed (median = 45 days)	Early: 31 (12–54) Deferred: 28 (10–56)	No difference in primary composite end-point. But early ART associated with low risk of progression to AIDS or death (OR = 0.51, 95% CI 0.27–0.94) and no increase in adverse events or IRD
Abdool Karim *et al.* [31••]	SAPIT; NCT00398996	South Africa	642	Smear positive pulmonary TB and CD4 cell counts <500 cells/μl	Early: 2 'integrated' arms started ART with first 3 months of TB treatment Late: the deferred group started ART within 1 month of the end of TB treatment	Integrated: 150 (77–254) Sequential: 140 (69–247)	The hazard of death in the early 'integrated' groups was 0.44 (95% CI 0.25–0.79) overall, 0.54 (0.30–0.98) in those with CD4 counts ≤200 cells/μl and 0.16 (0.03–0.79) in those with a CD4 count >200 cells/μl
Blanc *et al.* [33]	CAMELIA; NCT00226434	Cambodia	661	Smear positive pulmonary or extrapulmonary TB and CD4 cell counts <200 cells/μl	Early arm: within 2 weeks Late arm: after 2 months	Early: 25 (11–56) Late: 25 (10–55)	35% lower risk of mortality in the early arm
Torok *et al.* [30]	NCT00433719	Vietnam	253	Tuberculous meningitis	Immediate ART versus ART deferred for 2 months	Early: 39 (18–116) Late: 43.5 (16–84)	Hazard of death in immediate arm were 1.12 (95% CI 0.81–1.55; $P = 0.52$)
Makadzange *et al.* [32••]	NCT00830856	Zimbabwe	54	Cryptococcal meningitis	Early arm: within 72 h of diagnosis Late arm: after 10 weeks of treatment with fluconazole	Early: 27 (17–69) Late: 51.5 (25–69.5)	The hazard of death in the early arm was 2.85 (95% CI 1.1–7.23)

ART, antiretroviral therapy; IQR, interquartile range; OI, opportunistic infection; TB, tuberculosis.

Figure 1 Graph showing the probability of survival without death or development of an AIDS-defining illness in the randomized controlled trial comparing antiretroviral therapy initiation within 14 days of starting treatment for acute opportunistic infections (excluding tuberculosis) versus delayed antiretroviral therapy until after completion of the opportunistic infection



ART, antiretroviral therapy. Adapted from [29••].

treatment (sequential arm) [31••]. Patients with diagnoses of new or recurrent TB and with CD4 cell counts less than 500 cells/μl were included. The primary outcome was all-cause mortality (Table 2).

Following an interim analysis by the data safety and monitoring board, the sequential arm of the study was halted due to a high mortality rate. This initial report from the study compared the outcomes of patients in the two integrated arms combined with those of patients in the sequential arm [31••]. Overall, the hazard of death in the integrated arms was 0.44 (95% CI 0.25–0.79). For those with CD4 cell counts 200 or less or 200–500 cells/μl, the hazards of death were 0.54 (0.30–0.98) and 0.16 (0.03–0.79), respectively. Thus, delay of ART initiation until after the completion of TB treatment was associated with significantly higher mortality risk for all patients regardless of CD4 cell count stratum.

Study limitations included the enrolment only of patients with smear-positive pulmonary TB. Smear-negative and extrapulmonary TB are more frequent forms of disease at lower CD4 cell counts and are associated with higher mortality risk. The study was open-label and clinical judgement took precedence over the protocol-defined timing of ART, potentially undermining baseline randomization during follow-up. There were 36 withdrawals from the study and 41 patients were lost to follow-up, representing a total of 12% of patients enrolled.

This study has drawn criticism with regard to the inclusion of patients with CD4 cell counts less than 200 cells/μl into the sequential arm in which ART was deferred by upto 7 months for new TB cases and upto 9 months for retreatment TB cases [35,36]. It was predictable that such patients would have higher mortality and it is difficult to justify that equipoise existed when the study was designed in 2005. It was known that such patients had high case fatality rates of 16–35% during 6 months of TB treatment [37] and that ART substantially reduced mortality risk [38,39]. Moreover, a systematic review of TB IRD in 2005 found not a single case that resulted in death [8] and there were no data to indicate drug cotoxicity resulted in appreciable mortality.

Notwithstanding this ethical concern, these data provide important evidence that all TB patients with CD4 cell counts in the range 0–500 cells/μl should receive concurrent (integrated) TB treatment and ART. The key remaining question is the optimal timing of ART during the initial 2–3 months of TB treatment.

Pulmonary tuberculosis in Cambodia

More recent data provided by an open label RCT conducted in Cambodia (the 'CAMELIA' trial) have provided more precise data for the optimum timing of ART in TB patients with CD4 cell counts less than 200 cells/μl (Table 2) [33]. Eligible patients ($n = 661$) were ART-naive adults with newly diagnosed smear-positive pulmonary or extrapulmonary TB. ART initiation after 2 and 8 weeks of TB treatment were compared. Follow-up was for a minimum of 1 year (mean, 2 years). The primary outcome was death.

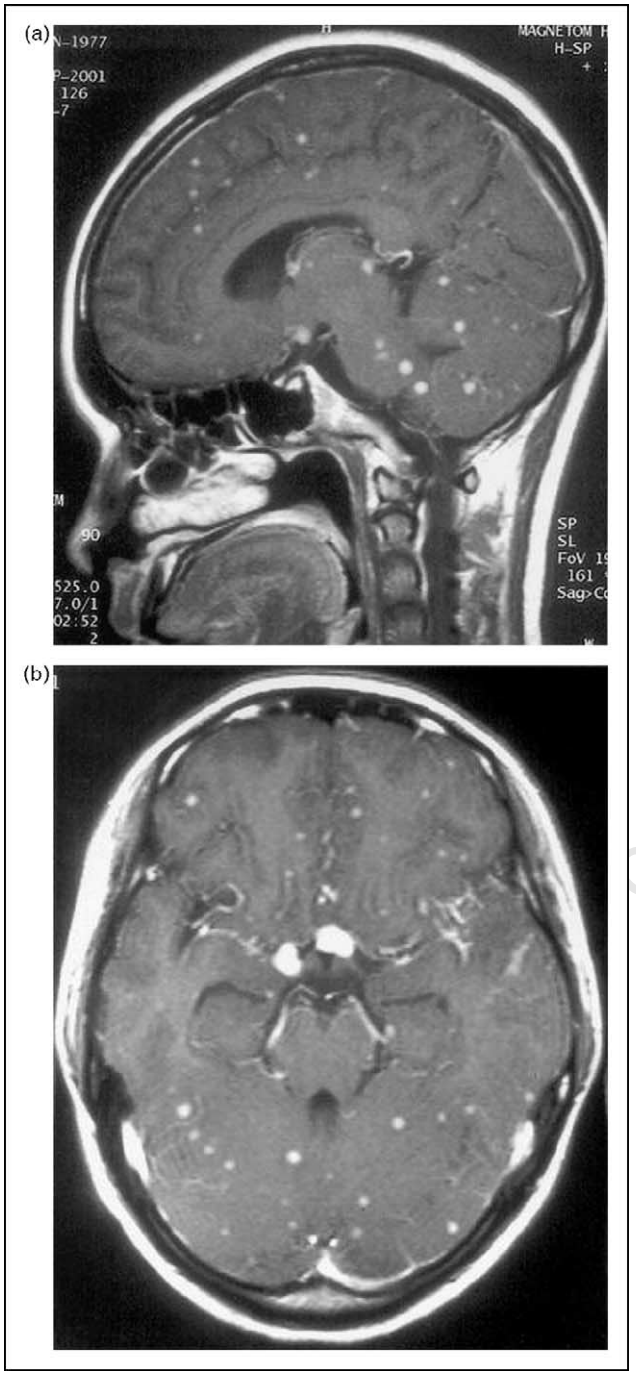
The mortality rate in the early arm was 8.3 deaths/100 person-years (95% CI 6.4–10.7) compared to 13.8 deaths/100 person-years (11.2–16.9) in the late arm ($P = 0.002$). IRD events were twice as frequent in the early arm (33 versus 15%) and, although there were five IRD deaths in the early arm compared to one IRD death in the late arm, this did not off-set the benefits of early ART. In adjusted analyses, the hazards of death in the late arm was 1.52 (1.12–2.05; $P = 0.007$) and early treatment was associated with a 35% lower mortality. Kaplan–Meier analyses showed that the survival benefit gradually accrued during long-term follow-up and was not a short-term effect. This was not related to long-term differences in the immunological and virological response to ART and the reasons for this remain unclear.

The benefit observed is entirely consistent with the data from Zolopa *et al.* [29••] as discussed above. However, it should be noted that in both these studies, patients had very advanced immunodeficiency and further data from patients with less advanced disease and from a range of settings are required.

Tuberculous meningitis in Vietnam

HIV-associated TB meningitis (Fig. 2) has a devastating prognosis, with a mortality of 67% and median time to

Figure 2 Saggital and transverse T1-weighted MRI scans



Saggital (a) and transverse (b) T1-weighted cranial MRI scans showing multiple tuberculomata in a patient with HIV-associated tuberculous meningitis.

death of 20 days reported from the cohort patients without ART availability in Vietnam [40]. The risks and benefits of early versus delayed ART are unknown and may differ from those in patients with other forms of TB. A randomized double-blind placebo-controlled trial of patients with HIV-associated TB meningitis ($n = 253$) has now been completed in Vietnam [30] (Table 2). The proportions with grade I, grade II and grade III meningitis were 32, 38 and 29%, respectively, and the median CD4 cell counts in the early and late arms were 39 and 44 cells/μl. Patients received a 9-month TB treatment that contained rifampicin throughout. Either efavirenz-based ART (immediate arm) or placebo (deferred arm) were started at the same time as TB treatment and all patients subsequently received open label ART from 2 months. Adjunctive corticosteroids tapered over 6–8 weeks and cotrimoxazole were also standard of care for all patients. Mortality at 9 months was the primary outcome.

Mortality was high at 9 months. Kaplan–Meier survival proportions were just 35.2 and 40.3% in the immediate and deferred arms, respectively. Compared to the deferred arm, immediate ART was not significantly associated with mortality (hazard ratio 1.12; 95% CI 0.81–1.55; $P = 0.50$) or the time to new AIDS events or death (hazard ratio 1.16; 95% CI 0.87–1.55; $P = 0.31$). The dominant independent predictor of mortality was the TB meningitis grade.

Grade III or IV adverse events were observed in both immediate and deferred arms during the course of the study (90 versus 89%), but these were more common during the first 2 months in the immediate arm (86 versus 75%, respectively; $P = 0.04$). Secondary end-points of immunological and virological responses to ART were, as expected, more rapid with immediate treatment. Overall, these data do not support immediate initiation of ART in patients with TB meningitis. Further data are needed from other settings.

Cryptococcal meningitis in Zimbabwe

Cryptococcal meningitis remains a major cause of HIV-associated morbidity. Mortality rates of 10–25% are reported from high-income settings but may be considerably higher in resource-limited settings [41]. Little, however, is known about the optimal timing of ART. A randomized open-label clinical trial conducted in Zimbabwe compared initiation of ART within the first 72 h of antifungal treatment with initiation after 10 weeks [32••]. All patients presented with their first episode of cryptococcal meningitis and were ART-naive. They were treated with oral fluconazole (800 mg once daily) and nevirapine-based ART and followed up for 3 years. The trial was discontinued prematurely after an interim analysis by the data safety and monitoring board found a significantly higher mortality in the early treatment arm.

The baseline characteristics of the two groups were adequately matched. However, the 3-year mortality in the early and deferred arms was 88 versus 54% ($P = 0.006$) and median survival in the two arms was 28 and 637 days, respectively ($P = 0.031$). In adjusted analyses, early ART was associated with an almost three-fold greater mortality

Table 3 Summary of ongoing trials examining timing of antiretroviral initiation in HIV-associated tuberculosis (TB)

Trial and sponsor	Setting and sample size	Type of tuberculosis	CD4 count at entry (cells/μl)	ART regimen	Treatment arms	Duration of follow-up	Primary outcome measure(s)
SAPIT (follow-up of arms 1 and 2): A study to compare three existing time points for starting ART in HIV/TB patients. Centre for the AIDS Programme of Research in South Africa (NCT00398996)	South Africa; $N=429$	Smear positive pulmonary TB	<500	Didanosine, lamivudine, efavirenz	Arm 1: ART within first 4 weeks Arm 2: ART within 4 weeks of completion of intensive phase	18 months	All cause mortality and progression to AIDS-defining illness
TB-HAART: An evaluation of the impact of early initiation of HAART on TB treatment outcomes for HIV/TB patients (ISRCTN77861053), WHO/TDR	South Africa, Uganda, Zambia, Tanzania; $N=1900$	Smear and culture positive pulmonary TB	220–500	Zidovudine, lamivudine, efavirenz	Arm 1: ART initiation at 2 weeks Arm 2: placebo for 6 months followed by ART	24 months	Composite endpoint of TB treatment failure or death at 6 months after initiation of TB treatment
ACTG A5221: Immediate versus deferred start of ART in HIV-infected adults being treated for tuberculosis (NCT00108862), NIAID	USA, Brazil, Haiti, South Africa, Kenya, Malawi, India, Thailand; $N=800$	Confirmed or probable TB	<200	Tenofovir, emtricitabine, efavirenz	Arm 1: ART initiation at 2 weeks Arm 2: ART initiation at 8 to 12 weeks	48 weeks	Survival without progression to AIDS
Anti-HIV Drugs for Ugandan Patients with HIV and Tuberculosis (NCT00078247) NIAID	Uganda; $N=350$	Smear or culture positive pulmonary TB	>350	Zidovudine, lamivudine, abacavir	Arm 1: immediate ART for 6 months Arm 2: ART delayed until CD4 count <250 cells/mm^3	2 years	CD4 count decline and progression to AIDS
TIME: Appropriate timing of HAART in co-infected HIV/TB patients (NCT01014481), Bamrasnaradura Infectious Diseases Institute	Thailand; $N=210$	Clinical or smear or culture positive TB	<350	Tenofovir, lamivudine, efavirenz	Arm 1: ART initiation after 4 weeks Arm 2: ART initiation after 12 weeks	144 weeks	Composite endpoint of death rate, hospitalization rate and adverse drug reactions
Simultaneous versus sequential ART and TB treatment (NCT00737724), Instituto Nacional de Enfermedades Respiratorias	Mexico; $N=160$	Active pulmonary TB	<200	Tenofovir, emtricitabine, efavirenz	Arm 1: immediate ART Arm 2: ART initiation after 2 months	96 weeks	Signs and symptoms of active tuberculosis; mycobacterial load in body fluids or affected tissues

ART, antiretroviral therapy; HAART, highly active antiretroviral therapy; TB, tuberculosis.

risk (adjusted hazard ratio 2.85; 95% CI 1.1–7.23). A large majority of deaths occurred in the first 2 weeks after diagnosis and almost all occurred within 4 weeks. Causes of death were ascribed using clinical observation and were primarily thought to be due to complications of cryptococcal meningitis.

The data show that under the circumstances studied, early ART was very strongly associated with higher mortality risk. However, a number of factors need to be considered when interpreting these data [42]. 'Early ART' was given very early indeed (<72 h) and 'late ART' was given very late (>10 weeks). Many clinicians would perhaps not intuitively choose either of these timings for treating their patients. Patients received high-dose oral fluconazole (a fungistatic drug) for their cryptococcosis as this is the standard of care in most African countries. Use of amphotericin, a fungicidal drug, is thought to clear cryptococcal antigen from the CNS more rapidly [41] and may therefore be associated with a lower risk of IRD, which was potentially a key factor driving mortality in the early arm. Patients were also under routine care of local hospital physicians rather than the study team and did not have protocol-driven monitoring and interventions to reduce intracranial pressure, a very important component of the management of this condition. There was neither clinical case definition nor management plan for cryptococcal IRD – a likely important cause of raised intracranial pressure and mortality [43]. Thus, the excess mortality risk associated with early ART might have been diminished by amphotericin use and appropriate management of raised intracranial pressure. Future studies will need to address alternative ART timing strategies, different antifungal regimens, management of raised intracranial pressure and use of adjunctive corticosteroids.

Current WHO guidelines

The WHO guidelines (2002) for the use of ART in adolescents and adults have been sequentially revised in 2003 and 2006 and most recently in 2010 [44•]. Data from early observational studies and later RCTs have resulted in a gradual shift in the guidelines towards earlier initiation of ART in those with TB. The most recent guidelines recommend that all patients with HIV-associated TB should start ART regardless of the CD4 cell count and this should be done as soon as possible within the first 8 weeks of TB treatment [44•]. Although the data from the CAMELIA trial emerged after publication of these guidelines, the data are nevertheless entirely supportive of the recommended timing [33]. No specific recommendations were made regarding the management of TB meningitis, however, but the currently available data [30] do not show an adverse impact of early treatment on survival as discussed above. No recommendations either were given in the current WHO guidelines regarding the timing of ART during treatment of other serious acute opportunistic infections. Future guidance will be particularly important with regard to the management of cryptococcal meningitis.

Ongoing clinical trials

A number of ongoing RCTs also aim to address the optimum time to start ART in TB patients (Table 3). These studies are being conducted in a wide range of geographical locations and include patients with differing degrees of immunodeficiency. A variety of study primary outcomes include survival, disease progression, TB treatment outcomes and drug toxicity. However, none of these studies includes participants under the age of 13 years and thus a critical need exists for studies in children. A further RCT (NCT01075152) was also funded in 2010 to investigate the optimal timing of ART in patients receiving amphotericin treatment for cryptococcal meningitis at three sites in Uganda and South Africa (D. Boulware, personal communication).

Conclusion

Great progress has recently been made in defining the optimum timing of ART in patients with serious opportunistic infections. The overall conclusion from the accumulated data is that early ART (within the first 2 weeks) is associated with lower mortality for patients with *P. jirovecii* pneumonia, serious bacterial infections and pulmonary TB compared to treatment at later time points. However, RCTs have shown that immediate ART conferred no survival benefit in patients with TB meningitis in Vietnam and was associated with substantially higher mortality risk in patients receiving fluconazole for cryptococcal meningitis in Zimbabwe. In both these conditions, further data are required to determine the optimum management strategies.

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Tubercle and Lung Disease

Pulmonary tuberculosis in HIV infection: radiographic appearance is related to CD4+ T-lymphocyte count

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S U M M A R Y. ***Setting:*** **An adult HIV outpatient clinic in Cape Town, South Africa.**
Objective: **To investigate the relationship between the radiographic appearance of pulmonary tuberculosis (PTB) in HIV infected patients and CD4+ T-lymphocyte count.**
Design: **Pretreatment radiographs of 150 patients with newly diagnosed PTB were reviewed. CD4+ T-lymphocyte count was used as a marker of HIV disease progression.**
Results: **Upper zone infiltrate typical of PTB reactivation was present in 18 patients. This pattern was associated with early HIV infection (mean CD4+ T-cell count 389) and had 78% positive predictive value for identifying patients with > 200 CD4+ T-lymphocytes/μL. Pleural effusion was present in 32 patients and occurred over a wide intermediate range of CD4+ T-cell counts (mean 185). Lower or midzone infiltrates, adenopathy, interstitial pattern or normal radiograph occurred in 136 patients and were associated with advanced HIV disease (mean CD4+ T-cell count 105). These patterns had 84%, 89%, 89% and 100% positive predictive value, respectively, for identifying patients with < 200 CD4+ T-cells/μL.**
Conclusion: **Pulmonary tuberculosis in African HIV-positive patients presents with a spectrum of radiographic abnormalities, and specific patterns are predictive of stage of HIV disease progression. In patients dually infected with HIV and PTB, chest radiographs are a useful adjunct to clinical staging.**

R É S U M É. ***Cadre:*** **Dispensaire ambulatoire pour des adultes VIH+ à Cape Town, Afrique du Sud.**
Objet: **Evaluer la relation entre l'aspect radiographique de la tuberculose pulmonaire (PTB) et le nombre de lymphocytes T CD4+ chez des malades VIH+.**
Schéma: **Les radiographies pré-traitement de 150 malades atteints d'une PTB nouvellement diagnostiquée ont été évaluées. Le comptage des lymphocytes T CD4+ a été utilisé comme marqueur de l'évolution de la maladie VIH.**
Résultats: **Un infiltrat des territoires supérieurs typique d'une réactivation de PTB était présent chez 18 des malades. Cette caractéristique était associée à une infection VIH précoce (numération moyenne des cellules T CD4+: 389) et avait une valeur positive prédictive de 78% dans l'identification des malades avec > 200 lymphocytes T CD4+/μL. Un épanchement pleural était observé chez 32 malades et survenait pour une large échelle intermédiaire de numération des cellules-T CD4+ (moyen 185). Des infiltrats des territoires inférieurs ou moyens, des adénopathies, des profils interstitiels ou des radiographies normales sont survenus chez 136 malades, en association avec une maladie VIH avancée (numération moyenne des cellules-T CD4+: 105). Ces profils avaient une valeur positive prédictive respectivement de 84%, 89%, 89% et 100% dans l'identification de malades avec < 200 cellules-T CD4+/μL.**
Conclusion: **La tuberculose pulmonaire chez des malades africains VIH+ se présente avec une gamme d'anomalies radiographiques; certaines images sont prédictives d'un stade de l'évolution de la maladie VIH. Chez des malades avec une double infection VIH/PTB, la radiographie thoracique représente une adjonction utile à la classification du stade clinique.**

R E S U M E N. ***Marco de referencia:*** **Dispensario para adultos VIH + en Ciudad del Cabo, Africa del Sur.**

Correspondence to: Dr Robin Wood, Department of Medicine, UCT Medical School, Observatory 7925, South Africa.

Paper received 12 January 1995. Final version accepted 24 March 1995.

Objetivo: **Evaluar la relación entre el aspecto radiográfico de la tuberculosis pulmonar (PTB) y el recuento de linfocitos T CD4+ en los pacientes infectados con VIH.**
Método: **Se revisaron las radiografías pre-tratamiento de 150 pacientes con PTB recientemente diagnosticada. Se utilizó el recuento de linfocitos T CD4+ como marcador de la evolución de la enfermedad VIH.**
Resultados: **En 18 enfermos se constató una zona infiltrada apical, típica de una reactivación de una PTB. Esta característica estaba asociada a una infección VIH precoz (recuento promedio de linfocitos T CD4+ : 389) y tenía un valor predictivo positivo de 78% para identificar pacientes con > 200 linfocitos T CD4+/μl. En 32 pacientes se observó un derrame pleural, el cual se presentaba en un amplio grado intermedio de recuento de linfocitos T CD4+ (promedio : 185). En 136 pacientes se observó un infiltrado en las zonas bajas o intermedias, adenopatías, sombras de tipo intersticial o radiografías normales, características que estaban asociadas a una enfermedad VIH avanzada (recuento de linfocitos CD4+ promedio : 105). Estos tipos de imágenes tenían un valor predictivo positivo para identificar pacientes con < 200 linfocitos T CD4+/μl, de 84%, 89%, 89%, y 100% respectivamente.**
Conclusión: **La tuberculosis pulmonar en los pacientes africanos VIH+ se presenta con un espectro de anormalidades radiográficas; ciertas imágenes específicas son predictivas del estado de evolución de la enfermedad VIH. En los enfermos con doble infección VIH/PTB, la radiografía de tórax representa un auxiliar útil para la evaluación del estado clínico.**

INTRODUCTION

The interaction between the human immunodeficiency virus (HIV) epidemic and pulmonary tuberculosis (PTB) has been recognised in both the developed and developing world. In sub-Saharan Africa, HIV seroprevalence of up to 67% has been reported among patients with newly diagnosed PTB.[1,2] The World Health Organisation (WHO) included PTB as one of the HIV clinical stage 3 criteria[3] and more recently it has been proposed that PTB should be an acquired immunodeficiency syndrome (AIDS) defining diagnosis.[4] In African HIV-infected patients, however, PTB was found to be a poor marker of stage of disease. In areas of high PTB prevalence, individuals may be HIV seropositive and develop PTB without significant immunosuppression.[5]

Patients dually infected with HIV and PTB frequently present with chest radiographic abnormalities that differ from those of PTB in HIV-negative individuals.[6–10] This study, conducted in an area with a high PTB prevalence of 1134/100 000[11] and an estimated 2.5% HIV seroprevalence,[12] investigated the relationship between various radiographic patterns and CD4+ T-lymphocyte counts.

METHODS

Medical records of adults attending Somerset Hospital HIV outpatient clinic in Cape Town, South Africa, from 1989–1994 were examined. Pretreatment chest radiographs of 150 patients (103 M, 47 F, mean age 33, range 17–75) in whom PTB was diagnosed were reviewed. These radiographs and those of 100 consecutive HIV clinic attendees without respiratory symptoms were reported on by a radiologist and categorized in a blinded fashion by 2 physicians independently. In case of disparity, the radiologist reviewed the films and was the final arbiter.

Radiographs with infiltrates localized predominantly in the upper lobes or apical segment of the lower lobes with or without cavitation were categorized as typical of reactivation (post-primary) PTB. In addition, radiographs were screened for the presence of pleural effusions, mediastinal and hilar adenopathy, lobar or segmental parenchymal infiltrates, and reticulo-nodular or miliary pattern. Fibro-cystic changes and pleural scarring were considered evidence of previous PTB. Patients without typical reactivation pattern could be included in more than one category.

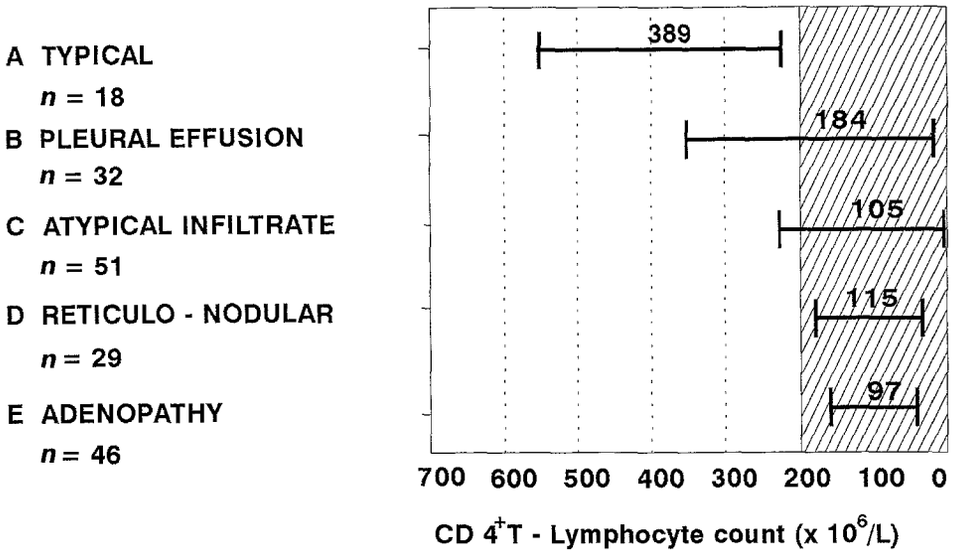
A PTB diagnosis was confirmed on sputum in 104 patients (smear only $n = 40$, smear and culture $n = 42$, sputum culture with negative smear $n = 22$). Pleural fluid adenosine deaminase (ADA) and pleural culture/histology ($n = 8$), effusion ADA only ($n = 9$), lymph-node histology ($n = 12$), blood culture ($n = 3$) or clinical and radiographic response ($n = 14$) to 4-drug antituberculosis medication (Isoniazid, Rifampicin, Ethambutol and Pyrazinamide) suggested active PTB. Smear-positive sputum was not routinely cultured. In accordance with WHO recommendations,[13] HIV seropositivity was confirmed by a combination of four positive ELISA assays on two separate blood samples. CD4+ T-lymphocyte counts were determined by flow cytometry.

Statistical analysis

Sensitivity, specificity and positive predictive values were calculated with standard formulae.[14] CD4+ T-lymphocyte counts were expressed as means ± standard deviation (SD). CD4+ T-cell counts associated with various radiographic patterns were compared by *T* test. A two-sided level of significance of 0.05 was used to reject null hypotheses.

RESULTS

The distribution of chest radiographic patterns of PTB in 150 HIV-positive patients and the mean CD4+



Figure—CD4+ T-Lymphocyte counts associated with PTB radiographic patterns in 150 HIV positive patients. CD4+ T-cell numbers are shown as mean ±SD. Pattern A is defined by upper zone infiltrate with or without cavitation and pattern C by parenchymal infiltrate in mid or lower zone. Pattern A is associated with early HIV disease and patterns C, D, and E with advanced HIV infection. Patients may be included in one or more of categories B–E.

T-lymphocyte count (±SD) associated with each pattern is shown in the Figure. Patients with pleural effusions had a significantly lower mean CD4+ T-lymphocyte count when compared to those with radiographs typical of reactivation PTB ($P < 0.01$). Adenopathy, lower or midzone parenchymal infiltrates and diffuse reticulo-nodular or miliary pattern were associated with significantly lower mean CD4+ T-cell counts than either pleural effusions ($P < 0.05$) or typical reactivation pattern ($P < 0.01$).

Radiographs with typical upper zone infiltrates with or without cavitation had 96% specificity and 78% positive predictive value for identifying subjects with > 200 CD4+ T-lymphocytes/μL. Cavitation occurred exclusively within upper zone infiltrates. Three patients had a normal chest radiograph, were sputum smear positive and had CD4+ T-lymphocyte counts < 200/μL. The specificity and positive predictive values for the atypical radiographic patterns for identifying patients with < 200 CD4+ T-lymphocytes/μL are shown in the Table. Apical fibrocystic disease and pleural scarring, representing prior PTB, had no predictive value for HIV stage of disease (data not shown) and occurred in 12% of both the patient and the control population.

Table: The specificity and positive predictive value of atypical PTB radiographic patterns for identifying HIV positive patients with < 200 CD4+ T-cells/μL

Radiographic pattern		Specificity	Positive predictive value
Normal radiograph	(*n* = 3)	100%	100%
Reticulo-nodular	(*n* = 36)	88%	89%
Adenopathy	(*n* = 46)	85%	89%
Lower/midzone Infiltrate	(*n* = 51)	76%	84%

Mycobacterial culture of sputum and pleural effusion was performed in 71 patients. *Mycobacterium tuberculosis* was the pathogen cultured in 94% (64 of 68 patients), three cultures lost viability or became contaminated. Atypical mycobacteria (*M. kansasii, M. xenopi* and two undefined non-tuberculous mycobacteria) were isolated from sputum of four patients who had < 200 CD4+ T-cells/μL. These patients were excluded from analysis. Sputum smear or culture positivity occurred in 73% of PTB patients with reticulo-nodular or miliary pattern. Of 100 HIV-positive PTB-negative controls, 75% had a normal chest radiograph, 12% showed evidence of previous PTB, 7% had parenchymal infiltrates and 6% a reticulo-nodular pattern. Hilar or mediastinal adenopathy was not present in these PTB negative patients and was thus not a feature of the persistent generalized lymphadenopathy of HIV infection.

DISCUSSION

This study documented that PTB occurred at all stages of HIV infection, and that there was a spectrum of chest radiographic presentation related to HIV stage of disease.

In southern Africa, latent *M. tuberculosis* infection has been reported to be present in the majority of adults,[1,2,15] and HIV seronegative adults typically develop reactivation PTB with post-primary infiltrates and cavitation.[10,15] This pattern in our HIV-positive patients was associated with early HIV disease as manifested by relatively preserved CD4+ T-cell counts. Pleural effusion, although regarded as a marker of early clinical HIV disease,[16,17] occurred throughout an intermediate range of lymphocyte counts and its presence was less helpful for prediction of HIV stage of disease.

In advanced HIV disease, both PTB reactivation and reinfection radiographically resemble primary tuberculosis, with features such as adenopathy and interstitial or non-cavitating parenchymal infiltrates.[16,17,18] These radiographic abnormalities occurred in our patient population despite historic or radiographic evidence of prior reactivation PTB. In contrast to miliary PTB in HIV-negative patients,[19] HIV-positive patients with reticulonodular and miliary pattern have multi-bacillary PTB[1] confirmed in our patients by frequent sputum smear and culture positivity. In agreement with previous reports, adenopathy was the best predictor of low CD4+ T-lymphocyte count.[17,20] A histopathological spectrum of *M. tuberculosis* infection in HIV disease analogous to that seen in *M. leprae* infection has been described.[1] This study reports a radiographic spectrum of pulmonary tuberculosis related to stage of HIV disease. In early HIV disease, a preserved cell mediated response results in paucibacillary PTB with apical fibrosis and cavitation. In advanced HIV infection, patients present with normal chest radiographs, interstitial patterns or adenopathy and advanced immunosuppression results in high bacterial load. The latter as radiographic appearances of PTB could be regarded as AIDS-defining criteria in our HIV-positive patients.

Although outpatient records were reviewed, patients had often required hospital admission or been referred from the community. The sample of patients might therefore be a reasonable representation of HIV-associated tuberculosis in our area. Tuberculosis in early HIV disease might have been under-represented, as specific abnormalities prompting HIV testing are often absent during early HIV infection. HIV serology is extremely sensitive,[21] and the multiple ELISA essays performed on our patients should ensure a high specificity. Consequently, the chance of false HIV seropositivity in our patients should be minimal.

In sub-Saharan Africa, PTB is the commonest opportunistic infection in HIV disease and CD4+ T-lymphocyte counts are frequently unobtainable. In this setting, radiographic criteria are a useful adjunct to clinical HIV disease staging. Prospective studies, however, will be required to confirm these findings in other population groups.

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Ultrasound for the diagnosis of HIV-associated tuberculosis

To the Editor: A recent letter in the *Journal*[1] suggested that ultrasound of the abdomen might be an appropriate way to diagnose tuberculosis (TB) when sputum smears are repeatedly negative for TB and in febrile patients known or suspected to be HIV-positive. The authors describe ultrasound findings they regard as pathognomonic of TB but do not give the number of scans performed or the yield of helpful scans. We have presented such an analysis with regard to microbiological investigations for TB,[2] and provide data for abdominal ultrasound.

In our case series of 141 consecutive HIV-positive patients with proven TB, seen between October 1994 and September 1996 at Somerset Hospital, Cape Town, 35 (25%) had an abdominal ultrasound. This high figure relates in part to a concurrent study at Somerset Hospital of abdominal pain in HIV-infected patients.[3] TB was found to be the leading cause of abdominal pain and our study focused on the diagnostic approach to TB, leading to overlap. However, while ultrasound was performed in 12 of 17 patients with TB and abdominal pain, the majority of scans were performed to look for disease processes other than TB that might have contributed to pyrexia. As an aside, all scans were normal or reflected the likely presence of TB — indicating that the use of ultrasound to exclude concomitant non-tuberculous pathology was not warranted.

To investigate whether ultrasound can be an appropriate tool in the diagnosis of TB we applied our previously published diagnostic algorithm to the 35 patients with ultrasound scans (Fig. 1, Table I). This approach is compatible with that of Emby and Hunter[1] who only advocate use of ultrasound if initial microbiological investigations are negative. The algorithm we originally published has been modified here by adding ultrasound of the abdomen as the diagnostic investigation of choice in patients with negative sputum smears who do not have pleural effusion or who are negative on lymph node biopsy.

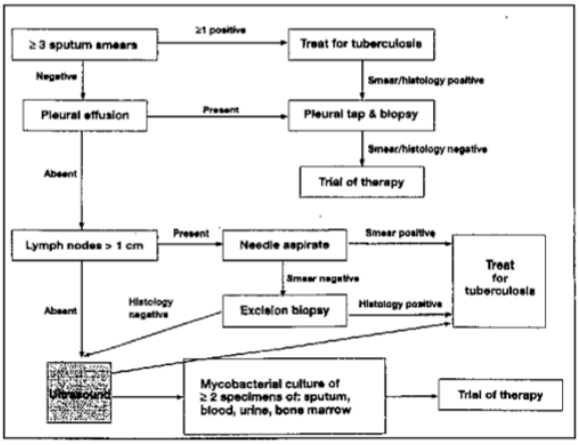

Fig. 1. Diagnostic algorithm for HIV-infected patients with suspected tuberculosis, modified from Hudson et al.[2] *to include abdominal ultrasound.*

Scans were classified as normal, reporting intra-abdominal lymphadenopathy, or abnormalities of the liver or spleen according to the radiologist's report. When a pericardial effusion was suspected on clinical grounds the request form for the abdominal scan also recorded the need to examine the heart in the manner suggested by Emby and Hunter. In 2 of the 10 patients with intra-abdominal lymphadenopathy the radiologist examined the heart and both scans demonstrated a pericardial effusion, confirming the clinical assessment. Of the 9 patients with other abdominal abnormalities 2 requests for ultrasonographic assessment of the heart yielded 2 further pericardial effusions. However, owing to the directed nature of these assessments we cannot comment on whether all abdominal scans should be accompanied by an assessment of the heart, as implied by Emby and Hunter. Many radiographers might be reluctant to assess patients in this way routinely.

Table I shows for each category of abdominal scan the

Table I. Eligiblity for scan had algorithm been followed in patients with HIV-associated tuberculosis, Somerset Hospital

	Abdominal ultrasound result		
	Normal (*N* = 16)	Intra-abdominal lympha-denopathy (*N* = 10)	Abnormal liver or spleen (*N* = 9)
Patient eligible (*N*)			
Diagnosed on urine culture	1	-	-
Diagnosed on sputum culture	-	1	1
Diagnosed on liver biopsy	-	-	1
Patient ineligible (*N*)			
Not anaemic (Hb > 11.0 g/dl)	2	-	-
Sputum smear positive	3	2	5
Peripheral nodes suitable for biopsy	10	7	1
Pleural effusion	-	-	1

number of patients for whom the scan was appropriate or not in terms of the diagnostic algorithm. It can be seen that the number of scans could have been reduced to 6. Two of the 16 negative scans could have been avoided if a policy was in force of only scanning patients with anaemia (a surrogate marker of dissemination).

In conclusion, there is a limited role for ultrasound in the diagnosis of TB. However, at a time of crisis in academic and rural medicine in South Africa, calls for use of existing investigations in new ways should be qualified by careful cost-benefit analysis. We should also point out that our study was performed in an HIV-positive population at very high risk of TB.[4] Experience elsewhere may differ, possibly resulting in ultrasound being even less useful.

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Massive hepatomegaly due to visceral leishmaniasis

To the Editor: In 1908 Leishman and Donovan[1] described the protozoan *Lesishmania donovani* in splenic tissue. Almost a century later leishmaniasis has emerged in new regions and new settings.[1] Recent interest in the disease has been prompted by recognition of cases in returning US Gulf War veterans and people with HIV infection.[2] We describe a case of visceral leishmaniasis diagnosed on bone marrow aspirate in a patient presenting to a tertiary hospital in KwaZulu-Natal.

A 42-year-old Mozambican national, who works as a marine merchant, was referred from a local hospital with fever, hepatosplenomegaly and pancytopenia. He had a 6-month history of gradual weight loss of approximately 10 kg with intermittent fever and rigors. He was treated for hepatic tuberculosis in a Mozambican hospital. A liver biopsy was not done before commencement of antituberculosis therapy and the patient did not improve after completion of this treatment.

He had been treated for malaria several years previously while living in Mozambique. He travelled to Brazil, Argentina, Italy and Portugal between 1995 and 1998.

On examination, the patient was febrile (39.8°C). He was pale and had no lymphadenopathy. He had a hepatomegaly that extended 10 cm below the costal margin and a 3 cm splenomegaly.

The full blood count showed haemoglobin 8 g/dl (normochromic, normocytic anaemia), platelets 132 × 10^9/l and white blood cell count 1.8 × 10^9/l. Liver function tests revealed a hyperglobulinaemia. He had an erythrocyte sedimentation rate (ESR) of 113 mm/h, and his urea and electrolytes were normal. HIV and hepatitis screens were negative. The chest radiograph was normal. Ultrasound of the abdomen detected no additional abnormalities. A bone marrow aspirate and trephine were done in the first instance to investigate the pancytopenia. A liver biopsy was scheduled, but this was deferred when the results of the bone marrow aspirate and trephine were received. Fig. 1 is a photomicrograph of the marrow aspirate showing the characteristic intracellular amastigotes of leishmaniasis.This was confirmed on the trephine biopsy.

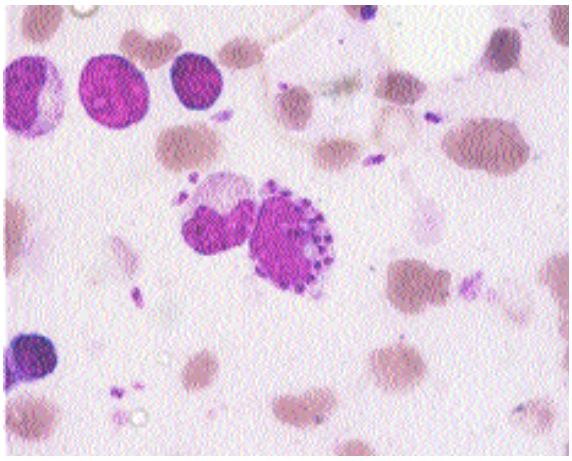

Fig. 1. Bone marrow aspirate — Gram stain showing intracellular 'dot-like' microorganisms (magnification 1 000). Courtesy of Dr P Sturm.

The patient was treated with intravenous amphotericin B (60 mg/kg/day) for 20 days. Renal impairment and thrombophlebitis complicated his therapy. The renal function improved when amphotericin B was stopped. At 3 months after treatment the hepatosplenomegaly had resolved and a repeat bone marrow aspirate was normal.

Discussion

This case highlights the importance of considering exotic diseases associated with common clinical presentations, given

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Utility of interferon-γ ELISPOT assay responses in highly tuberculosis-exposed patients with advanced HIV infection in South Africa

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Abstract

Background: Interferon-gamma (IFN-γ) ELISPOT assays incorporating *Mycobacterium tuberculosis*-specific antigens are useful in the diagnosis of tuberculosis (TB) or latent infection. However, their utility in patients with advanced HIV is unknown. We studied determinants of ELISPOT responses among patients with advanced HIV infection (but without active TB) living in a South African community with very high TB notification rates.

Methods: IFN-γ responses to ESAT-6 and CFP-10 in overnight ELISPOT assays and in 7-day whole blood assays (WBA) were compared in HIV-infected patients (HIV+, n = 40) and healthy HIV-negative controls (HIV-, n = 30) without active TB. Tuberculin skin tests (TSTs) were also done.

Results: ELISPOTs, WBAs and TSTs were each positive in >70% of HIV- controls, reflecting very high community exposure to *M. tuberculosis*. Among HIV+ patients, quantitative WBA responses and TSTs (but not the proportion of positive ELISPOT responses) were significantly impaired in those with CD4 cell counts <100 cells/μl compared to those with higher counts. In contrast, ELISPOT responses (but not WBA or TST) were strongly related to history of TB treatment; a much lower proportion of HIV+ patients who had recently completed treatment for TB (n = 19) had positive responses compared to those who had not been treated (11% versus 62%, respectively; P < 0.001). Multivariate analysis confirmed that ELISPOT responses had a strong inverse association with a history of recent TB treatment (adjusted OR = 0.06, 95%CI = 0.10–0.40, P < 0.01) and that they were independent of CD4 cell count and viral load. Among HIV+ individuals who had not received TB treatment both the magnitude and proportion of positive ELISPOT responses (but not TST or WBA) were similar to those of HIV-negative controls.

Conclusion: The proportion of positive ELISPOT responses in patients with advanced HIV infection was independent of CD4 cell count but had a strong inverse association with history of TB treatment. This concurs with the previously documented low TB risk among patients in this cohort with a history of recent treatment for TB. These data suggest ELISPOT assays may be useful for patient assessment and as an immuno-epidemiological research tool among patients with advanced HIV and warrant larger scale prospective evaluation.

Background

The HIV-associated tuberculosis (TB) epidemic in sub-Saharan Africa is fuelling a global increase in TB incidence of 1% per year [1]. TB incidence rates in southern Africa have reached almost unprecedented levels [2] and much of this disease remains undetected in the community [3]. This escalating epidemic led to the declaration by the World Health Organisation in 2005 of "a regional emergency requiring urgent and extraordinary actions" [4]. However, most existing tools with which to confront the TB epidemic are blunt, especially those used for diagnosis of *Mycobacterium tuberculosis* infection and disease in HIV-infected patients.

Recent developments of immune-based assays to detect *Mycobacterium tuberculosis* infection are a significant advance [5]. ESAT-6 and CFP-10 are two proteins encoded by the RD1 genomic segment of *M. tuberculosis*, which is absent from all BCG strains and the vast majority of environmental mycobacteria [6-8]. As a result, enzyme-linked immunospot (ELISPOT) assays that detect interferon-gamma (IFN-γ) release in response to these antigens differentiate between *M. tuberculosis* infection and immune sensitisation by BCG vaccination or exposure to environmental mycobacteria. In outbreaks of TB in the UK, ELISPOT responses among contacts showed better correlation with the degree of exposure than tuberculin skin tests (TSTs) [9,10]. Among HIV-negative patients with culture-positive TB, ELISPOT assays have a sensitivity of approximately 80–90% [11-13]. Moreover, increasing evidence suggests that ELISPOT responses in human and bovine models correlate with mycobacterial load during antituberculosis treatment [13-18].

At present, very few studies have examined the utility of ELISPOT assays in HIV-infected individuals. In a study from Zambia, ELISPOT responses to ESAT-6 or CFP-10 were positive in 90% (n = 39) of HIV-infected patients with sputum smear-positive pulmonary TB [19]. Also, when used in the diagnosis of TB in South African children, the sensitivity of the assay was not significantly impaired by HIV coinfection [12]. More recently the assay was found to be relatively unimpaired in the detection of either latent *M. tuberculosis* infection or active TB in patients with moderately advanced HIV infection [20,21]. However, responses in those with advanced HIV have not previously been reported.

The aim of the present study was to identify determinants of ELISPOT responses among patients with advanced HIV infection (but not active TB) living in a South African community with very high TB incidence. Overnight IFN-γ ELISPOT assay responses were assessed among a group of HIV-infected patients enrolling in an antiretroviral treatment service and were compared with responses in a group of healthy controls living in the same community. To provide greater insight, these responses were compared to 7-day whole blood assays (WBA) of IFN-γ release and TSTs.

Methods

HIV-infected (HIV+) patients were recruited to the study at the antiretroviral treatment clinic based at the Gugulethu Community Health Centre in Cape Town. The study cohort and clinic have previously been described in detail [22-25]. The district has a predominantly African population of over 300,000, the vast majority of whom live in conditions of low socioeconomic status. In 2003 the antenatal HIV seroprevalence was 28% and the annual TB notification rate exceeded 1,000/100,000 [26]. Patients were referred to the ART programme for evaluation for eligibility for antiretroviral treatment under national guidelines.

Consecutive HIV+ patients were recruited to the study before starting antiretroviral treatment. Non-pregnant adults between the ages of 18 and 50 years, who were not currently receiving antituberculosis treatment nor had evidence of active TB were eligible. Details of previous medical history were obtained from the referral letter and were cross-checked with the patient. Patients were clinically characterised and carefully evaluated for TB. Available investigations for TB included sputum smear microscopy and liquid culture (MGIT, Becton Dickinson, Sparks, Maryland, USA), chest radiology, and fine needle aspiration of lymphadenopathy for cytology and culture. Nebulised sputum induction was accessible when required. Patients with active TB diagnosed at baseline were excluded as were those who subsequently developed TB within 4 months of the recruitment date since they may have had active sub-clinical TB at the time of testing. Standard

antituberculosis treatment nationally uses a 6-month rifampicin-containing regimen and primary and secondary isoniazid prophylaxis is not recommended by the national TB control programme.

Healthy HIV- control patients aged 18–50 years were recruited from the same community. These individuals were part of a prospective cohort being studied in preparation for phase III HIV vaccine trials. Consecutive individuals with two negative HIV tests three months apart who remained asymptomatic and had no previous history of TB or other significant morbidity were enrolled.

At a single clinic visit venous blood was taken and TSTs were done using 2 TU purified protein derivative (PPD) RT23 (Statens Serum Institut, Copenhagen, Denmark) on the volar aspect of the forearm. TSTs were read by a single health care worker experienced in the technique, measuring the diameter of induration at 48–72 hours using callipers. Plasma HIV-1 load was measured using Versant™ HIV-1 RNA 3.0 branched chain DNA assay (Bayer HealthCare, Leverkusen, Germany) and blood CD4 cell counts were measured by flow cytometry using FACSCount™ (Becton Dickinson Inc., Franklin Lakes, NJ, USA). All subjects studied gave written informed consent. The study was approved by the Research Ethics Committee of the University of Cape Town and conformed to the declaration of Helsinki.

ELISPOT assays

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized venous blood by Ficoll-Paque centrifugation. A commercially available IFN-γ ELISPOT kit containing a pre-coated 96-well plate was used (Mabtech, Stockhom, Sweden). Paired wells with 250,000 cells/well were stimulated with anti-CD3 antibody at 100 ng/ml (positive control, Mabtech), were left unstimulated (negative control) or contained the following antigens each at 5 μg/ml final concentration: PPD RT49 (Statens Serum Institut) or recombinant ESAT-6 and CFP-10 (Lionex, Braunschweig, Germany). After incubation for 18 hours, plates were developed according to the manufacturer's protocol. Plates were read on an Immunospot Series 3B Analyzer (Cellular Technology, Cleveland, OH, USA) and were retained for visual inspection in the case of anomaly. Low level spot counts in unstimulated negative control wells were subtracted from the test well results. The mean number of spot-forming units (SFU) in paired wells was multiplied by 4 to provide SFU/10^6 PBMC. Counts of ≥ 20 SFU/10^6 PBMC that were at least ≥ 2-fold greater than background counts were predefined as positive responses. Using this cut-off, this assay produces very similar results to other commercially available and 'in-house' assays performed in the same laboratory [20,21].

Whole blood assays

Whole blood assays were done using a methodology similar to that described previously [27,28]. Heparinized whole blood was diluted 5-fold using RPMI 1640 supplemented with penicillin, streptomycin and 2 mM L-glutamine and was plated into 96-well plates in the presence of antigen or mitogen or left unstimulated. Phytohaemagglutinin (PHA, Sigma, St. Louis, MS, USA), PPD RT49, and recombinant ESAT-6 and CFP-10 were all used at a final concentration of 5 μg/ml. Any small residual traces of endotoxin in antigen preparations were neutralised using 10 μg/ml polymyxin B sulphate (Sigma) as verified in pilot experiments. Day 7 supernatants were harvested following incubation at 37°C. All assays were piloted and conditions, antigen concentrations and timepoints were optimised using patient and control samples. IFN-γ responses to recombinant ESAT-6 were equivalent when compared with those generated using a sample of recombinant antigen from Statens Serum Institut, Copenhagen.

Concentrations of IFN-γ were determined in paired samples of supernatant using an ELISA with a standard curve that ranged from 10,000 pg/ml to 41 pg/ml and using previously described methodology [28,29]. Background concentrations of IFN-γ were subtracted from sample results, which were then categorised as positive if ≥2-fold higher that the lower limit of detection of the assay. Negative control values falling below the lower limit of detection were assigned half the value of the lowest standard.

All laboratory assays were done blinded to the status of the patients.

Data analysis

χ^2, Fisher's exact and Mann-Whitney U tests were used to compare proportions and medians as appropriate. Assay responses were evaluated as continuous (quantitative) variables and as categorical variables (positive or negative responses) according to the thresholds stated above. Associations between patient characteristics and positive ELISPOT and WBAs were examined using univariate analyses and multiple logistic regression models. CD4 cell count, plasma viral load and history of previous TB were included in the multivariate models *a priori*; age and sex were included if there was a trend towards an association ($p < 0.10$). SAS version 8.2 (SAS, Cary, North Carolina, USA) and Prism version 4.0 (GraphPad Software, San Diego, CA, USA) software were used for data analysis.

Results

Patient characteristics

A total of 77 subjects were recruited; 7 were excluded due to failed ELISPOT assays (n = 4; no positive control response or technical error) or the development of possi-

ble TB (n = 3). Eleven individuals invited to participate declined (predominantly due to transportation difficulties). Among those included in the analysis, 40 were HIV+ patients being evaluated for antiretroviral treatment; these had baseline characteristics (Table 1) which were similar to those described in previous reports of this treatment cohort [22-24]. None of the patients had serious co-morbidity (such as lymphoma, Kaposi's sarcoma requiring cytotoxic chemotherapy or diabetes mellitus) that might have affected T cell assays. CD4 cell counts showed that immunodeficiency was advanced in most patients; 48% had a history of completed treatment for TB within the preceding 3 years (Table 1) consistent with previous findings [22]. The median period since completion of TB treatment was 4 months (IQR, 2–13 months). HIV- controls (n = 30) had similar age and sex distribution as HIV+ patients (Table 1).

A large proportion (58%) of the HIV+ patients had cutaneous anergy to PPD (Table 1) and TSTs reactions >10 mm occurred in a significantly lower proportion of HIV+ patients than HIV- controls (43% versus 77%, $P < 0.05$). TST responses were significantly associated with CD4 cell count; the proportion of patients with a TST response >10 mm was much lower among those with CD4 cell counts <100 cells/μl (n = 17) compared to those with CD4 cell counts >100 cells/μl (n = 23) (16% versus 56%, respectively; $P < 0.05$). In contrast, TST responses were not significantly associated with history of TB treatment ($P = 0.2$).

ELISPOT and WBA results

In all 70 subjects included in the analysis the positive control stimuli produced measurable responses in ELISPOT assays and WBAs. The magnitude of responses to all stimuli were significantly lower among HIV+ patients compared to HIV- controls (Table 2). However, in contrast to TSTs, the magnitude of these responses in HIV+ patients was not significantly associated with CD4 cell count using either assay. Instead IFN-γ responses, especially ELISPOT results, were strongly associated with history of previous TB treatment; responses among treated patients were lower than those who had no history of treatment (Table 2).

We next calculated the proportions of patients with assay responses categorised as positive according to the predefined thresholds (Figure 1). In general, greater proportions of 7-day WBA responses were positive compared to the overnight ELISPOT assay. Furthermore, the proportions of positive responses were typically greater using PPD compared to RD1 antigens.

Positive responses to RD1 antigens were detected in 70% of healthy HIV- controls using the ELISPOT assay and in a significantly greater proportion using the 7-day WBAs (93%; $P < 0.05$) (Figure 1). Positive responses to PPD were also observed in very high proportions of HIV- controls using both assays (Figure 1). Similar to the quantitative responses (Table 2), the proportions of positive ELISPOT responses in HIV+ patients were also very strongly associated with previous history of TB treatment (Figure 1). Compared to those who had not previously received TB treatment, treated patients had a 5.6-fold lower proportion of positive ELISPOT responses to RD1 antigens ($P < 0.01$) and a 2.4-fold lower proportion of positive ELISPOT responses to PPD ($P = 0.02$) (Figure 1). In marked contrast, WBA responses in HIV+ patients did

Table 1: Characteristics of HIV-negative controls (HIV-) and HIV-infected patients (HIV+)

Characteristic	HIV- controls	HIV+ patients		
		All	With history of TB	With no history of TB
Number	30	40	19	21
Median age (years)	28	31	30	32
Female	23 (77)	32 (80)	13 (68)	19 (90)
CD4 count (cells/μL)				
Median (IQR)	-	114 (72–246)	98 (44–160)	117 (77–267)
Distribution				
0–99	-	17 (43)	10 (53)	7 (33)
100–199	-	10 (25)	5 (26)	5 (24)
≥200	-	13 (32)	4 (21)	9 (43)
Median viral load		5.0 (4.5–5.3)	5.0 (4.6–5.4)	4.9 (4.4–5.3)
TST (mm)				
0	6 (20)	23 (58)	13 (68)	10 (48)
1–9	1 (3)	0 (0)	0 (0)	0 (0)
≥100	23 (77)	17 (43)	6 (32)	11 (52)

Unless otherwise stated, values show numbers (%) of patients.
Viral load in log copies/mL. TST = tuberculin skin test

not show a significant inverse association with history of TB treatment (P > 0.25 for each comparison) (Figure 1). Thus, there was a clear dissociation between ELISPOT responses and WBA responses in those who had previously been treated for TB (Figure 1).

We next examined the effect of CD4 cell count on the proportion of positive assay responses by comparing the results of those with CD4 cell counts <100 cells/μl (n = 17) with those of patients with CD4 cell counts >100 cells/μl (n = 23). There was no significant difference between these groups using the WBA (82% vs 61%, respectively; P = 0.18) although there was a non-significant trend towards an association using the ELISPOT assay (24% vs 52%, respectively; P = 0.10). Importantly, however, history of TB treatment is confounded as a variable by CD4 cell count in this cohort [22] (Table 1). Thus, we next analysed ELISPOT responses stratified by both CD4 cell count and history of TB treatment. This clearly showed that responses were strongly associated with history of TB treatment but not with CD4 cell count (Figure 2).

To confirm these findings we next did multivariate analysis to identify factors independently associated with positive ELISPOT responses to RD1 antigens (Table 3). This showed that ELISPOT responses were not significantly associated with CD4 cell count or viral load but that there was a highly significant independent association with history of TB treatment.

Responses in HIV+ patients with no history of TB treatment

Results thus far indicated that HIV+ patients who did not have a history of recent TB treatment were much more likely to have positive ELISPOT responses to RD1 antigens than those who had been treated. We reasoned that while treated patients were likely to have cleared any viable mycobacteria, untreated patients would have a similar risk of latent *M. tuberculosis* as HIV- controls. We therefore compared IFN-γ responses in HIV- controls and the subgroup of HIV+ patients with no history of TB treatment. There was indeed no significant difference between these groups in quantitative ELISPOT responses to PPD, ESAT-6 and CFP-10 (P > 0.1 for all comparisons) whereas quantitative WBA responses were much lower in the HIV+ subgroup (P < 0.01 for all stimuli) (Table 2). Moreover, the proportion of positive ELISPOT responses did not significantly differ between the HIV- and the untreated HIV+ subgroup (P = 0.55) (Figure 2).

Table 2: Quantitative interferon-γ (IFN-γ) responses to anti-CD3 (positive control) and mycobacterial antigens as assessed by ELISPOT assay and 7-day whole blood assay (WBA)

Patient characteristics	ELISPOT[a]				WBA[b]			
	Anti-CD3	PPD	ESAT-6	CFP-10	PHA	PPD	ESAT-6	CFP-10
HIV- (n = 30)	1723 (846–2471)	61 (26–214)	24 (7–199)	30 (6–89)	>10,000	>10,000	4820	3678
HIV+ (n = 40)	626 (398–2015)	21 (2–53)	7 (2–46)	6 (0–39)	3991	799	355	96
P value	0.012	0.001	0.004	0.010	<0.001	<0.001	<0.001	<0.001
HIV+ CD4>100[c] (n = 23)	654 (290–2296)	30 (4–64)	16 (2–58)	30 (0–82)	4213	616	34	49
HIV+ CD4<100 (n = 17)	600 (400–1912)	8 (2–49)	2 (1–17)	1 (1–10)	2550	1034	528	125
P value	0.93	0.42	0.19	0.12	0.13	0.89	0.14	0.12
HIV+ no previous TB Rx[d] (n = 19)	476 (269–2299)	34 (3–147)	22 (2–80)	36 (1–80)	6938	1529	495	179
HIV+ previous TB Rx (n = 21)	1020 (400–1844)	6 (2–42)	5 (0–14)	0 (0–6)	2749	313	65	20
P value	0.70	0.085	0.044	0.028	0.10	0.115	0.220	0.038

[a]ELISPOT responses expressed as median (interquartile range) spot-forming units/10^6 peripheral blood mononuclear cells.
[b]WBA responses expressed as median values in pg/mL.
[c]CD4 cell count in cells/μL.
[d]TB Rx = treatment for tuberculosis

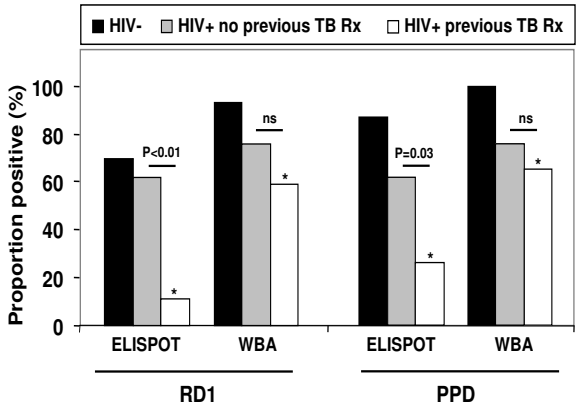

Figure 1
Proportion of positive IFN-γ ELISPOT responses and 7-day IFN-γ whole blood assay (WBA) responses to ESAT-6 and/or CFP-10 (RD1) and purified protein derivative (PPD) among HIV- controls (n = 30) and HIV+ patients (n = 40) subdivided into those with (n = 19) or without (n = 21) a history of previous tuberculosis treatment (TB Rx). ELISPOT assay responses in HIV+ patients were strongly associated with history of TB treatment. *In HIV+ patients who had previously received TB treatment, the proportions of positive responses to RD1 and PPD antigens were significantly lower using the ELISPOT assay than the WBA (P < 0.05 for each comparison). ns = not significant.

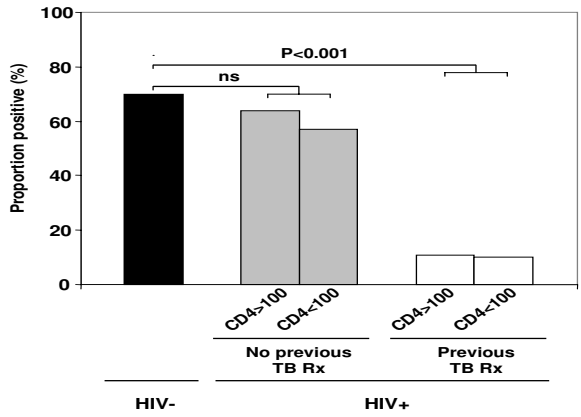

Figure 2
Proportion of positive ELISPOT IFN-γ responses to *M. tuberculosis*-specific antigens (ESAT-6 and/or CFP-10) among HIV- controls (n = 30) compared to HIV+ patients (n = 40) stratified by CD4 cell count >100 cells/μl (n = 23) or <100 cells/μl (n = 17) and according to history of recent TB treatment (n = 19) or no history of such treatment (n = 21). ns = not significant

Discussion

This study conducted in a setting with very high TB burden is, to our knowledge, the first to assess the utility of ELISPOT responses to *M. tuberculosis*-specific antigens among HIV-infected patients with advanced immunodeficiency. To provide further insight into the determinants of such responses, we compared the overnight ELISPOT assay (which assesses rapid effector memory T cell responses [18,30,31] with a 7-day IFN-γ WBA (which reflects central memory T cell responses [32]. We found that both assays were able to detect IFN-γ responses to ESAT-6 and CFP-10 in a substantial proportion of HIV+ patients despite the low median CD4 cell count of 114 cells/μl. The proportion of positive ELISPOT responses was independent of CD4 cell count in stratified and multivariate analyses (Table 2, Figure 2); instead responses were strongly associated with history of TB treatment (Figures 1 and 2; Tables 2 and 3). Almost half the HIV+ patients had completed treatment for TB a median of 4 months earlier, resulting in likely clearance of the burden of viable mycobacterial infection; this was reflected by a low proportion of positive ELISPOT responses in these patients but persistence of a high proportion of positive

Table 3: Multivariate analysis of factors associated with positive IFN-γ ELISPOT responses to ESAT-6 or CFP-10 in HIV+ patients (n = 40)

Variable		Crude OR	P value	Adjusted OR	P value
Age	<31 years	1.00		-	-
	>31 years	0.58 (0.16–2.12)	0.41	-	-
Sex	Male	1.00		-	-
	Female	5.44 (0.60–49.56)	0.13	-	-
CD4 count	>100	1.00		1.00	
(cells/μL)	<100	0.34 (0.08–1.34)	0.12	0.58 (0.11–3.16)	0.53
Log viral load	<5.0	1.00			
(copies/mL)	>5.0	0.39 (0.10–1.49)	0.17	0.30 (0.05–1.69)	0.17
History of TB	No	1.00			
	Yes	0.07 (0.01–0.40)	<0.01	0.06 (0.10–0.40)	<0.01

OR = odds ratio

WBA (memory) responses. In contrast, ELISPOT responses in HIV+ patients with no history of TB treatment did not significantly differ from those of HIV- controls. Collectively, these observations suggest the ELISPOT assay provides data that are clinically and immuno-epidemiologically informative in patients with advanced HIV.

Antigen stimulation in 7-day WBA permits T cells to differentiate and proliferate, leading to amplification of IFN-γ responses. Similar to lymphocyte proliferation assays, WBAs thereby provide an assessment of memory responses to antigens encountered at any time past or present. In contrast, the overnight ELISPOT assay detects rapid effector memory cells; such cells are thought to have recently encountered antigen from viable organisms *in vivo* and can rapidly release IFN-γ when re-exposed to antigen *in vitro* [18,30,31]. Thus, ELISPOT responses appear to be more strongly associated with current antigen load rather than with past antigen exposure [13-18]. These facts provide a logical rationale for the very strong inverse association between ELISPOT responses (but not 7-day WBA responses) and history of recent TB treatment in HIV+ patients.

The HIV- control group was recruited in the same community and had similar demographic characteristics as the HIV+ group and so were likely to have similar prior exposure to *M. tuberculosis*. Retention within an HIV-negative cohort for ≥3 months provided the opportunity to prospectively verify that these patients were free of both TB and HIV. Positive WBA responses to mycobacterial antigens were detected among a very high proportion of HIV- controls, which is consistent with extremely high rates of previous exposure to *M. tuberculosis* in this community. Similar to a study in a neighbouring community in Cape Town [20], TSTs ≥ 10 mm and ELISPOT responses to RD1 antigens were positive in 70% and 77% of HIV- controls, respectively, consistent with high rates of latent *M. tuberculosis* infection.

The HIV+ group was drawn from a very well-characterised cohort of patients enrolling for antiretroviral treatment in which risk factors for TB have been established [22]. An important finding previously reported was that patients who had completed successful TB treatment within the preceding 2 years had an approximately 5-fold lower risk of active TB compared to those who had not [22]. The ELISPOT results in this study strongly concur with this observation. We suggest that successful TB treatment would have cleared viable mycobacterial infection, resulting in low risk of reactivation TB and negative ELISPOT responses due to absence of antigen. An alternative hypothesis to explain the ELISPOT data would be that those who had previously had TB developed lasting suppression of IFN-γ responses to RD1 antigens, although the WBA data do not support this.

Our findings are consistent with previous studies showing that HIV infection does not appear to substantially undermine ELISPOT responses in patients with either active TB [12,19,21] or latent *M. tuberculosis* infection [19-21]. In two separate studies, Rangaka *et al*. found that assay responses to RD1 antigens were not significantly impaired in two cohorts of patients with latent infection and moderate HIV-associated immunodeficiency (both median CD4 cell counts of 392 cells/μl and 464 cells/μl) [20,21]. Our data extend this observation to patients with advanced immunodeficiency (median CD4 cell count = 114 cells/μl). Although we found no impact of CD4 cell count on the proportion of positive responses, there was a trend towards overall quantitative ELISPOT responses being reduced in those with CD4 cell counts <100 cells/μl and the study lacks the statistical power to adequately assess this.

The normalised input of peripheral mononuclear cells used in ELISPOT assays may, in part, explain why this assay appears to retain utility among patients with low CD4 cell counts. This also might explain why studies using a commercially available data overnight IFN-γ whole blood assay (QuantiFERON-TB Gold, Cellestis, Melbourne, Australia) in which the cell input is not normalised have found that responses do not appear to be greatly affected in HIV-infected patients with moderate immunodeficiency but that there was a high rate of indeterminate results among those with advanced immunodeficiency [20,33,34]. It cannot be assumed that only CD4 T cell responses are measured by these assays as other IFN-γ-secreting PBMC subsets such as CD8 T cells and NK cells may also be detected. Future immunophenotypic studies would be useful in this regard.

A high proportion of HIV+ patients had anergic TSTs, confirming the known limited utility of this test in advanced HIV. Moreover, both TST responses and quantitative WBA responses to mycobacterial antigens were very strongly associated with CD4 cell count, contrasting with the ELISPOT assay. A potential reason for this is that both TST and WBA responses depend on interleukin-2-mediated T cell proliferation, which is markedly inhibited by HIV infection [35,36]. In contrast, the overnight ELISPOT assay detects terminally differentiated effector cells [18,30] and is therefore independent of T cell proliferation.

This study has certain limitations. Case control studies may be subject to selection bias. However, the characteristics of the HIV+ group were representative of those previously described in this cohort [22] and HIV- controls had similar demographic characteristics as HIV+ patients and

were enrolled within the community rather than at health facilities. The assays used are research tools and are not directly comparable with commercially available IFN-γ-release assays currently used in clinical practice. Although the group of HIV+ patients with a history of TB treatment was heterogeneous with regard to the time since completion of TB treatment, we have previously found that such patients have a substantially lower risk of TB [22]. Thus, history of previous TB treatment was included as a key variable within the analyses. This study, however, was not designed to specifically evaluate the impact of TB treatment on ELISPOT responses and the data should not be over-interpreted in this regard. Common to all such studies, the sensitivity of the ELISPOT assay in diagnosis of *M. tuberculosis* infection in this population cannot be definitively evaluated in the absence of a gold standard. The study included few HIV-infected patients with high CD4 cell counts and this may have limited the ability to fully evaluate the effect of CD4 cell count on ELISPOT responses. However, our findings are consistent with other published data [20,21]. The number of patients studied was limited, but in view of the very high rates of TB exposure in this community and very strong statistical associations observed, the aims of the study were nevertheless achieved.

Conclusion

This study provides evidence that the IFN-γ ELISPOT assay retains utility among patients with advanced HIV infection as suggested by the following key observations: (i) the proportion of positive responses was not associated with CD4 cell count in stratified and multivariate analyses; (ii) responses were strongly related to history of TB treatment – a known key factor associated with TB risk in this patient population; (iii) responses in HIV+ patients who had not been treated for TB in the past were similar to those of HIV- controls. Collectively these data indicate that the ELISPOT assay provides data that are informative in patients with advanced HIV and is therefore likely to be useful for patient assessment and as an immuno-epidemiological tool. These data provide an important basis for the justification of future large scale evaluation of the assay in prospective cohort studies of patients with advanced HIV.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

SDL designed the study, analysed the data and wrote the manuscript. NB and MV did the laboratory analyses. MN obtained clinical data and did the TSTs. LGB, HMD and RW helped in the interpretation of data and helped revise the manuscript. All authors read and approved the final draft.

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CHALLENGES OF TB DIAGNOSIS AND TREATMENT IN SOUTH AFRICA

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It is estimated that 2 billion of the world's population are latently infected with *Mycobacterium tuberculosis* (Mtb) with a resultant 8 - 9 million cases of active tuberculosis (TB) and 1.6 million deaths annually.[1] The tools used for diagnosis of TB have remained largely unchanged since the 1880s when sputum microscopy, Mtb culture on solid media, tuberculin skin testing and chest radiology were initially developed. In 1991 the World Health Assembly set targets to be reached in 2005 for 70% case finding of smear-positive TB, which represents 6 million cases to be identified per annum.[2] A second target was that 80% (5 million) of those identified cases should complete anti-TB treatment.[2] Subsequently the millennium development goals of 2000 set a target of halving the prevalence of TB disease from 300/100 000 to 150/100 000 and deaths from 30/100 000 to 15/100 000 by 2015.[3] While progress toward these targets was being made in countries with established market economies there was a quadrupling of TB incidence between 1990 and 2005 in most African countries. In 2005 the World Health Organization Regional Committee for Africa declared tuberculosis an emergency for the African region.[4]

In South Africa in 2005 the WHO estimated that of 284 592 TB cases 270 360 were notified to the national TB control programme, representing a somewhat ambitious reported case finding proportion of 95%.[5] The proportion treated under the directly observed treatment (DOTS) programme is 94%, and HIV prevalence among notified cases was 58% (97.5% confidence interval (CI) 49 - 65%). South Africa is a middle-income country and is relatively well provided with 143 laboratories performing sputum smears, and 18 culture laboratories also capable of performing drug sensitivity testing.[5] Multidrug resistance (MDR) in new TB cases varies between provinces from 0.9% to 3.6%, while MDR is higher amongst retreatment cases, with prevalence rates varying between 1.8% to 13.7% in different provincial surveys.

THE CHALLENGES OF HIV TO TB CONTROL

The HIV epidemic in South Africa has been associated with a similar increase in TB case load as reported in other sub-Saharan countries. The incidence of TB has increased markedly among HIV-infected individuals, with a proportional increase in smear-negative pulmonary disease, extrapulmonary involvement, frequent atypical clinical presentations, an increased mortality and a strong association with multidrug and extreme drug-resistant TB. The impact of HIV on a TB clinic is illustrated by the changes in TB notification data from a Cape Town peri-urban township over the last 10 years as HIV adult seroprevalence has increased from 8% in 1996 to 23% in 2005.[6] During this period TB incidence rates have increased 4.75-fold from 400/100 000 in 1996 to 190 000/100 000 in 2005, with the highest increase occurring in 20 - 40-year-olds.[6] In 2005 the overall TB notification rate was 5.4-fold higher among HIV-positive individuals (5 140/100 000) than HIV-negative individuals (953/100 000) (Table I). Smear-negative disease notification was 8-fold higher among HIV-positive individuals (1 891/100 000) than HIV-negative individuals (238/100 000). Despite a plateauing of HIV seroprevalence, TB notifications have continued to increase.[6]

THE CHALLENGES OF TB IN AN ART PROGRAMME

The diagnosis of TB is particularly challenging in patients accessing antiretroviral therapy (ART) when their HIV infection is advanced. The Hannan Crusaid clinic was the first

TABLE I. ADULT PULMONARY TB NOTIFICATION RATES PER 100,000 IN A CAPE TOWN PERI-URBAN TOWNSHIP 2005

TB type	Total adult population	HIV-positive adults	HIV-negative adults	HIV+ve/HIV -ve ratio
(PTB)	1 931	5 140	953	5.4
Smear-positive PTB	1307	3 248	715	4.5
Smear-negative PTB	624	1 891	238	7.9

PTB = pulmonary tuberculosis.

dedicated public sector ART facility in South Africa and currently provides treatment to 3 000 patients. Eighty-nine per cent of those accessing ART have symptomatic HIV disease (WHO clinical stage 3 and 4) with a median CD4 cell count of 95 cells/µl. More than 50% have a history of prior completed TB treatment, 15% are on current TB treatment, 11% are diagnosed with previously undiagnosed TB, and a further 10% develop new incident TB after initiation of ART.[7] Multivariate analysis identified risk factors for development of incident TB to be WHO stage 3 and 4 disease (relative risk (RR) 5.9, 95% CI 3.2 - 10.9 and 8.9 95% CI 4.6 - 17.3 respectively), baseline CD4 cell count (RR 1.41, 95% CI 1.2 - 3.1 for each 50 CD4 cell count decline) and baseline viral load (RR 1.4, 95% CI 1.1 - 1.8). A history of completed TB treatment within the previous 2 years was associated with significant protection against incident TB (RR 0.21, 95% CI 0.2 - 0.7).

THE CHALLENGE OF HIV/TB IN A COMMUNITY

The high case finding proportion (close to 100%)[2] reported for the South African TB control programme is based on an estimate of the TB burden. The programme is based on passive case finding together with directly observed therapy of those cases identified. Active case finding enables a direct assessment of TB burden and can identify differing case finding proportions for either HIV-negative or HIV-positive individuals. Active TB case finding and HIV testing of a randomly selected sample of 762 individuals living in Masiphumelele, a periurban township outside Cape Town, was performed in 2005 and identified 23% of adults to be seropositive for HIV, 11 individuals with prevalent treated TB and a further 12 individuals with previously unrecognised smear-positive ($N = 6$) and culture-positive ($N = 6$) pulmonary TB.[8] Both HIV infection and a history of recent incarceration were strongly associated with TB. The TB prevalence among HIV-infected individuals was 7.6%, of which 4.4% was smear positive disease. The case finding proportion for HIV-negative individuals (ratio of prevalence of treated to prevalence of treated and untreated with smear-positive disease) was 67% (95% CI 41 - 100), while that for HIV-positive individuals with smear-positive disease was 37% (95% CI 25 - 53) (Table II). In this community, with a single TB clinic providing care to the whole community, the TB control programme appeared to perform less well for those with HIV infection than for those who were HIV negative.

THE CHALLENGE OF MULTI- AND EXTREMELY DRUG-RESISTANT TB

In 2005 an outbreak of extremely drug-resistant TB (XDR) was recognised in Tugela Ferry, situated in a rural area of northern KwaZulu-Natal. A report of the first 53 cases was published in 2006.[9] The epidemic was recognised in predominantly HIV-positive individuals and was characterised by an extremely high early mortality rate. Over 50% of cases of XDR died within 30 days of presentation. Eighty per cent of the identified cases had positive sputum smears and 25% had

TABLE II. PREVALENCE OF TREATED AND UNTREATED SMEAR-POSITIVE PULMONARY TB WITH ACTIVE CASE FINDING AMONG HIV-SEROPOSITIVE AND HIV-SERONEGATIVE INDIVIDUALS

	HIV-positive adults	HIV-negative adults
Prevalence of treated smear-positive PTB	1 563 (1 108 - 2 138)	352 (233 - 507)
Prevalence of treated and untreated smear-positive PTB	4 400 (3 619 - 5 299)	527 (280 - 711)
Case finding proportion	0.37 (0.25 - 0.53)	0.67 (0.41 - 1.0)

PTB = pulmonary tuberculosis.

evidence of extrapulmonary involvement. Analysis of risk factors for XDR identified that 55% had no history of prior treatment for TB, indicating that these cases had primary rather than acquired resistance. Only 15% had a history of treatment default or failure. The one factor which was of concern was that 67% gave a history of admission to the local health facility, raising the concern that nosocomial transmission may have played a significant role in the epidemic. XDR cases have continued to be recognised, and 266 cases have been confirmed of which 264 were HIV-positive. This epidemic illustrates the potential problems associated with integration of HIV and TB programmes and the increasing need for rapid diagnosis of initial TB infection in both HIV-positive and negative individuals and recognition of early treatment failure and development of resistance within a treatment programme.

CURRENT TB DIAGNOSTICS

Current TB diagnostics, TB skin testing (TST), sputum smear and culture and radiology have remained the mainstay of TB diagnostics since 1882. TST has been used to support the diagnosis of TB in populations where TB infection is low. In South Africa a positive TST can be used to support the diagnosis in paediatric TB but is of limited use in adult diagnosis. TST is however still a useful tool for epidemiological studies of the annual risk of infection in children but by adulthood the majority of South Africans have been exposed to TB. In industrialised countries the TST identifies a minority of individuals who are at increased risk of disease progression, to whom diagnostic and preventive resources can be targeted. In contrast, in South Africa the majority of adults have already been exposed to TB and in those with advanced HIV infection, who are at highest risk of disease progression, the test has low sensitivity with up to 50% false negatives.

Sputum smear microscopy has high specificity in high TB prevalence settings. Sputum microscopy has been the mainstay of TB control programmes as it is able to identify the most infectious cases. The test is relatively inexpensive and widely available. Despite its wide availability in the field it is dependent on highly motivated technicians. The overall sensitivity for identifying TB infection is 35 - 70%, but the sensitivity in HIV-infected cases can be as low as 20%.

Diagnosis of TB with a chest X-ray is fast, convenient and has high sensitivity in HIV-negative individuals. In HIV infection the radiological findings of active TB disease decrease with the level of immune suppression, resulting in low sensitivity in advanced disease.[10] The occurrence of opportunistic diseases that can also mimic the radiological changes of TB results in lowered specificity in AIDS cases. The combination of relative expense, restricted availability and low sensitivity and specificity in HIV infection limits the role of radiology for TB control in the high HIV prevalence setting.

Mtb culture is sensitive and specific for pulmonary TB in both HIV-positive and HIV-negative individuals. Solid media culture, however, is limited by the prolonged time required for positive and negative results, which can be delayed for 6 - 8 weeks. Culture of sputum has low sensitivity for extrapulmonary TB, which occurs more commonly in HIV-infection. TB culture also requires a high level of laboratory bio-safety, which consequently restricts its availability to sophisticated centralised reference laboratories.

NEW TECHNOLOGIES

Serological diagnosis of TB is a relatively simple and inexpensive technology which could be a potentially attractive strategy for paediatric and adult extrapulmonary TB diagnosis. A large number of commercially marketed antibody detection tests are available. In 2005 the WHO performed an evaluation of available TB rapid diagnostic antibody tests. Nineteen of 27 invited manufacturers agreed to submit their products for evaluation against a panel of known specimens. The evaluation study found that the performance of antibody tests varied widely with high 'lot to lot' and 'reader to reader' variability. The specificity was less than 80% in the majority of products. Those tests with higher sensitivity lacked specificity and detected fewer than 40% of TB cases. The conclusion of the study was that none of the antibody assays performed well enough to replace microscopy.[11]

Liporabinomannan (LAM) is a component of Mtb cell walls, which is excreted unchanged in urine. A quantitative enzyme-linked immunoassay (ELISA) has been used to demonstrate a correlation between urinary LAM concentrations and the level of Mtb organism load in the sputum of pulmonary TB cases.[12] Urinary LAM concentrations may therefore be a reflection of infecting Mtb organism load, able to diagnose TB in both HIV-positive and HIV-negative individuals. The quantitative thresholds of urinary LAM for diagnosis and sensitivity, and specificity for identifying pulmonary and extra-pulmonary TB, are still to be defined. The LAM ELISA format is suited for peripheral laboratory use; however, a simpler 'tube format' test has also been shown to be robust and does not require a cold chain. A dipstick format of the test is under development, which could possibly prove be the first TB diagnostic suitable for use in 'point of care' clinics.

Cytokine detection assays are based on the observation that lymphocytes with immunological memory produce interferon-gamma (INF-γ) when re-exposed to a specific antigenic challenge. Two commercially available assays have been developed; the QuantiFERON®-TB GOLD, which uses whole blood as its substrate, and the T-SPOT TB assay, which uses isolated peripheral blood mononuclear cells. Both assays use Mtb specific antigens and therefore should not be subject to cross-reactions due to exposure to other environmental mycobacteria or exposure to the *M. bovis* strain used for BCG vaccination. The performance criteria of these tests for identifying latently infected individuals in published studies is in the range of 0.75 - 0.95 for sensitivity and 0.9 - 1.0 for specificity; however, the sensitivity in HIV-infected individuals may be reduced.[13] The advantage of INF-γ assays over TST, of a single visit with a result not subject to observer error, is offset by the requirement of venepuncture, the cost and the need for laboratory infrastructure. Although cytokine assays appear to be more sophisticated and sensitive versions of the TST, these tests may be measuring differing aspects of the immune responses to Mtb infection. INF-γ secretion by cells incubated with mycobacterial antigens over a week may reflect long-term immunological memory, while the shorter 3-day incubation may reflect more recent immunological memory. We have shown that the 3-day INF-γ secretion from PBMCs co-incubated with Mtb antigens is similar in both HIV-negative and HIV-positive controls, but the secretion from cells of those with a history of recent TB treatment was significantly lower than controls (Lawn *et al* in press).

CULTURE TECHNIQUES

Culture of Mtb remains the gold standard for both diagnosis and drug sensitivity testing. The characteristics of culture and media and growth detection are shown in Fig. 1. Culture in liquid media is faster, with results available as soon as 7 days compared with 42 - 56 days' required growth on solid media. A variety of growth detection methodologies have been utilised. Early detection of growth may be based on mycobacterial metabolism, identification of microscopic colonies or macroscopic plaques on secondary organisms. The BACTEC 460-TB® commercial assay incorporates a radioactive marker in the liquid media which is detected when growth occurs. A more recent development, the BACTEC MGIT 960® assay, utilises a plastic tube containing a broth with a fluorescence quenching-based oxygen sensor. Consumption of oxygen by growth of Mtb produces fluorescence when illuminated by a UV lamp. The non-commercial microscopic optical detection system (MODS) uses an inverted microscope to detect characteristic tangles of developing Mtb colonies in 96 well pates. The FAST-plaque™ assay identifies viable organisms which have been infected with bacteriophages by the development of macroscopically visible plaques on a lawn of fast growing *M. smegmatis*.[14]

Drug sensitivity testing (DST) conventionally takes 4 - 6 weeks after confirmation of primary detection on solid media, a process which takes 6 - 8 weeks. Liquid culture media can incorporate antibiotics at the time of initial inoculation, with the inference than surviving organisms are phenotypically resistant to the specifically incorporated antibiotic. DST results

Culture

- More sensitive than direct sputum smear, requires between 5 and 100 organisms/ml v. 5 000 - 10 000 for positive smear
- Allows species identification and concomitant drug sensitivity testing (DST)

Media

- Solid media requires 6 - 8 weeks for confirmed diagnosis and further 4 - 6 weeks for DST
- Liquid culture is faster with results of diagnosis and DST as soon as 7 days

Growth detection methods

- Radioactivity-BACTEC 460-TB®
- Fluorescence-BACTEC MGIT 960®
- Phage-based tests- FASTPlaque TB-RIF™
- Inverted microscopy-MODS

Fig. 1. Characteristics of Mycobacterium tuberculosis *culture.*

can therefore be available within the same time frame as mycobacterial diagnosis.

NUCLEIC ACID AMPLIFICATION TESTS

Nucleic acid amplification (NAA) is a rapidly evolving improvement in the detection and identification of Mtb which requires strong laboratory capacity and good quality control procedures and is relatively expensive. Bacterial or ribosomal RNA transcribed into DNA is amplified, followed by an appropriate reading system using a signal generating probe. The inclusion of internal positive controls reduces the incidence of false negatives, and use of a single tube format can reduce potential for contamination. NAA tests can be used for tuberculosis diagnosis but cannot be used for evaluation of patients receiving therapy as the technology cannot distinguish between live and dead organisms. The outstanding feature of NAA tests is that a positive result together with a high degree of specificity can be achieved within hours.

NAA tests usually have high specificity but variable sensitivity, so a positive test is good evidence of infection but a negative test is less informative. It is considered that current NAA tests cannot replace microscopy or culture, are unsuitable for smear-negative disease, and should be used only in conjunction with these tests and clinical data.[15,16] Drug resistance can be identified by identifying sequences in the rpoB and katG genes which encode for rifampicin and isoniazid resistance or by hybridisation of this region with specific DNA probes. Recent advances in NAA technology include the ability to amplify directly from clinical samples, isothermal amplification of DNA and improved amplification product detection.

The AMPLICOR® MTB test can give a result within 6 - 7 hours and is a US Federal Drug Administration (FDA) approved test for confirming smear-positive pulmonary TB LAMP (loop mediated isothermal amplification) is able to amplify TB DNA directly from clinical samples and does not require a thermocycling device, and a positive result, confirmed by a colour reaction visible with the naked eye, can be achieved within 2 hours. With further development a version of the LAMP test may be suitable for use in peripheral laboratories. A commercial assay (Hain Lifesciences) allows a specific Mtb diagnosis together with detection of rifampicin and isoniazid resistance achieved by PCR amplification of the 16S-23S ribosomal DNA spacer region followed by hybridisation of the amplified DNA product with specific oligonucleotide probes. The probes are immobilised as parallel lines on membrane strip however the test format is suited to reference laboratories rather than lower resourced peripheral laboratories.

DIAGNOSTIC PIPELINE

There is now more interest and financial investment in the development of new TB diagnostics than has occurred over the prior decades (Fig. 2). The development pipeline is active; however, most of the immediate advances in diagnosis and drug sensitivity testing will be applicable only to the reference laboratory.[17] Advances in peripheral laboratory capacity are 2 - 5 years away and are characterised by improved microscopy techniques and development of simplified nucleic acid amplification methodologies. There is little immediate hope of improved 'point of care' TB diagnostics, where the need is greatest. The time frame for new tests in the peripheral clinic is 3 - 7 years and is dependent on formulating simplified antigen and antibody testing such as dipstick tests. The role of newer diagnostics with potential to address the spectrum of clinical scenarios posed by TB and HIV infection are shown in Table III.

TABLE III. TESTS WITH POTENTIAL TO ADDRESS DIFFERENT CLINICAL SCENARIOS

Clinical scenario	Potentially useful tests
TB infection	
HIV negative	TST, INF-γ whole blood assays
HIV positive	TST, INF-γ isolated PBMC assays
TB disease	
Smear positive	Direct smear, rapid Mtb culture, phage, NAT,
Smear negative	Rapid Mtb culture, urinary LAM
TB treatment failure	Rapid Mtb culture with DST
Drug sensitivity testing	Rapid Mtb culture, phage assay, NAAT, hybridisation assays

CONCLUSIONS

The HIV epidemic has reversed the advances made in global TB control. The increase in smear-negative disease has exposed the inadequacies of existing diagnostics which have remained essentially unchanged since the 1890s. HIV infection has been associated with decreased sensitivity of all present diagnostic modalities of TST, sputum microscopy, TB culture and radiology. Active community case finding indicates that the present diagnostic algorithm used in the national TB control programme fails to identify a large proportion of HIV-associated tuberculosis. There is therefore an urgent need for

Target timetable	2007	2008	2009	2010	2011	2012
Reference Laboratory  "Faster than culture"	MGIT-960 diagnosis & DST Phage tests for rifampin resistance	Manual molecular DST	Manual NAAT resistance screen	Automated NAAT Real time PCR		
Peripheral Laboratory  "More sensitive than sputum smear"		Fluorescent microscopy	First generation LAMP	Urinary NAAT		
Clinic Based  "As simple as a dipstick"			Urinary antigen dipstick		Dipstick antibody test test	Second generation LAMP

*FIND = Foundation for Innovative New Diagnostics, NAAT = nucleic acid amplification test; DST = drug sensitivity test; LAMP = loop-mediated isothermal amplification.

Fig. 2. FIND time table for availability of new Mtb diagnostics.*[17]

point of care tests with increased sensitivity for screening of HIV-positive individuals.

The association of HIV-infection with MDR and XDR has also driven the need for tests which can be used to recognise early treatment failure and rapid identification of drug resistance. The rapid progression of TB in HIV-infected individuals together with increased mortality has also emphasised a need for much faster identification of infection and failure of therapy. The development of rapid liquid culture assays and NAA assays offer significant advances over conventional solid media culture and drug sensitivity testing.

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SAMJ
Letters to the Editor

Briewe

Importance of full disclosure of all medical conditions

To the Editor: We appeal to all medical practitioners to assist the insurance industry by completing the medical documents for insurance purposes fully, providing the company with accurate details and dates of all the applicant's medical conditions. An absence of accurate detailed information leads to increased costs by requiring further investigation to establish the severity of the condition.

As a general principle, the industry relies on full and accurate disclosure by the client of all relevant medical information so that the application can be underwritten fairly and equitably. The contract of assurance is one of good faith, which imposes a duty of full disclosure on the client. It is important that the client and the sales intermediary understand the consequences of material non-disclosure on the contract of assurance. Material non-disclosure will result in the contract's being voided or offered with a loading.

The insurance industry would greatly appreciate the assistance of the medical profession in achieving better levels of disclosure. I would like to record the industry's gratitude for the services that the medical profession provides to the insurance industry.

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'Discriminatory' advertising in the SAMJ

To the Editor: The April edition of the *SAMJ* included an advertisement for doctors for 'men's clinics' in different centres in the country. When I contacted Mr Nic Goslar to enquire about these positions I was told firmly that they were not interested in women doctors, as these clinics were for men with sexual problems. When I pointed out that it was no longer permissible to discriminate on the grounds of gender Mr Goslar laughed, admittedly rather uncomfortably. I hope that the *SAMJ* does not condone this type of discriminatory recruitment.

Bridget L Farham
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Mr Goslar replies: Thank you for bringing this matter to my attention. I hope that I have not offended Dr Farham by implying that the positions are only open to male doctors. I would like to point out, for the benefit of Dr Farham, that our group of clinics is in no way associated with the *SAMJ*, and that the *SAMJ* has never, as far as we are aware, condoned discriminatory recruitment.

It can't be done!

To the Editor: I wonder how many of us read Adam Smith's letter' and really took heed of its contents. How many of us reflected on the paragraph in which he quoted John Ruskin: 'the common law of business balance prohibits paying a little and getting a lot — it can't be done'.

The so-called 'full MASA rate' for medical practitioners was determined by the MASA in consultation with financial experts. It is *not* a fee determined to create wealth. The MASA fee ensures a reasonable return for the financial outlay of a practice and for the work and involvement with all its ethical and legal implications, and, *most importantly*, ensures that the medical professional can provide for his or her retirement years. Ask any 55-year-old doctor if he will be able to retire in 5 - 10 years' time. It will almost certainly be impossible! Any company director, on the other hand, is comfortably looking forward to his pension and retirement package.

Remember that the doctor who retires from practice does not have an asset to provide for the retirement years. I often tell my patients that my practice is a 'depreciating asset'. The goodwill is myself. It leaves when I leave, and my equipment is worth a pittance on the open market. The doctor needs to have secured a reasonable nest-egg, which cannot be done while desperately trying to make ends meet on a month-to-month basis. Stop fooling yourself with the Scale of Benefits fee. In order to survive financially you would need to create volume, and in the medical context this means over-servicing, or packing extra patients into your day (which is in any case impossible for certain specialists, such as anaesthetists). You cannot practise honest and decent medicine at these rates — it just can't be done.

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Patients who can't afford to pay — time for a code of ethics

To the Editor: Is it not time for private providers to introduce a code of ethics for dealing with patients who cannot afford to pay for their health services? Options for collaboration between the public and the private sector are being tossed back and forth, and mechanisms for regulating the private sector are on the table. But these processes take time, and it is surely better for the private sector to moderate its own activities than to wait for government intervention.

My concern arises out of a number of experiences — either first-hand or described to me — in which private health providers appear to have acted unethically, or at least against the best interests of patients who could not afford to pay for their health care.

As interns, one saw numerous patients who had been turned out of private hospitals once their medical aid cover had run out.

I remember tending to a street vendor whose femoral artery had been transected by a stray bullet in an armed robbery in the centre of Durban. We were inadvertently taken to a private hospital, where it was established that she could not afford to pay for the surgery. Precious time ticked by while we attempted to secure transport and a public sector destination which would undertake the limb-saving surgery.

Another incident was related to me by a perplexed and troubled mother, who could not understand why her son, seriously injured in a car accident, had been transferred 80 km for radiological procedures and treatment when these were available in a nearby state hospital. It turned out that the services were offered by private providers, and were available only to private sector patients over weekends. After being shuttled between hospitals and waiting over 18 hours, the young man was finally admitted to intensive care in a public hospital. He died 4 days later of adult respiratory distress syndrome. Did the delay in treatment contribute to his death?

I was told about the transfer of a medically unstable patient from a private hospital to a public hospital at 2 o'clock in the morning because he 'would have incurred a whole day's extra fees if he had stayed till morning'.

These are just a few of the disconcerting incidents of which I am personally aware. People's lives and limbs are being jeopardised because they cannot afford to pay for health care, particularly when, in an emergency, fate delivers them to the door of a private doctor or hospital.

Arguably, there are constitutional imperatives which would require private doctors to treat people in an emergency, regardless of their ability to pay. But surely more compelling are ethical considerations? At the moment it would seem that for some private providers, money is the only factor determining emergency management of the poor.

It seems to me that a code of ethics is urgently needed for the management of patients who can't afford to pay. This code should address at least: (*i*) emergency management of patients with life- or limb-threatening injuries; (*ii*) procedures for transfer from private to public health services; and (*iii*) care for chronic or long-term patients who have exhausted their medical aid cover.

There is general agreement that greater co-operation between the public and private sectors is needed if human resources for health are to be used more optimally. A private sector code of ethics for managing patients who cannot afford to pay would be a good place to start.

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Trace elements and osteoporosis

To the Editor: Wynchank and Saltman[1] suggest that trace elements may be of importance in the treatment and prevention of osteoporosis. Osteoporosis is multifactorial in origin, although the primary cause in postmenopausal women is oestrogen lack. Dietary supplementation with calcium, vitamin D and possibly trace elements and other factors may have a 'permissive' role in the prevention and treatment of osteoporosis, particularly in older subjects.[2] Many dietary factors have been claimed to have an effect on bone mass, including the trace minerals zinc, copper and manganese, as well as boron and silicon and also vitamins B_6, B_{12}, C and K.[3] Saltman and Strauss[4] compared the effect of dietary supplementation with: (*i*) calcium alone; (*ii*) trace elements alone; (*iii*) calcium and trace elements combined; and (*iv*) a placebo on bone mineral density (BMD) in four groups of postmenopausal women. The BMD fell in all the groups except those given calcium and trace elements combined. Oestrogen was only given to 35% of the women and the authors state that the groups were balanced in respect of the number of subjects receiving oestrogen therapy. According to the authors the oestrogen users neither lost nor gained significant quantities of bone. No information, however, was given about the non-users of oestrogen who, in all probability, experienced a significant fall in BMD. It is possible that trace elements may have a 'permissive' function, similar to that of calcium, in the prevention and treatment of osteoporosis in postmenopausal women. Much research, however, would be required to elucidate the role of supplementation with the different elements, particularly in different women with different dietary intakes. Trace elements are fairly ubiquitous and many calcium preparations used for dietary supplementation contain them in varying quantities. There is no evidence that elements such as zinc, copper and manganese, when taken in trace quantities, do any harm; in the manufacture of calcium preparations it may be easier, and less costly, to retain rather than remove such elements. The inclusion of trace elements in calcium supplements may not be undesirable, though more evidence is required to determine their importance in bone maintenance. Oestrogen replacement is the primary therapy in the prevention and treatment of postmenopausal osteoporosis.

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HIV infection is not associated with an increased rate of drug-resistant tuberculosis

To the Editor: Concern has been raised about an increased prevalence of primary and acquired tuberculosis drug resistance among HIV-positive patients.[1] No drug resistance data stratified for HIV status are available for the Western Cape, the region with the highest TB prevalence and incidence rates in South Africa.[2]

All 385 patients with newly diagnosed tuberculosis admitted to Somerset Hospital, Cape Town, from November 1994 until October 1996 were prospectively evaluated. An HIV test result was obtained in 90% (347 of 385); 50 patients were previously known to be HIV-positive. Of the 297 patients with TB and prior unknown HIV status, 115 (39%) tested positive for HIV-1. The prevalence of HIV infection was significantly higher in black patients than in coloured patients with TB (53% v. 37%, $P < 0.01$), being 42%, 54% and 80% in patients with pulmonary TB, tuberculous pleural effusion and extra-pulmonary TB, respectively. Tuberculosis in these hospitalised patients was associated with advanced HIV infection (median CD4 T-cell count 65/µl for pulmonary or extra-pulmonary TB and 172/µl for pleural TB).

Mycobacterial culture and drug sensitivities were available for 99 of 165 HIV-positive and 115 of 182 HIV-negative patients. All HIV-negative patients were infected with *Mycobacterium tuberculosis*, whereas 6 HIV-positive patients (6.1%) had atypical mycobacterial species isolated.

Sensitivity patterns of *M. tuberculosis* were as follows:

	HIV-positive	*HIV-negative*
MTB fully sensitive	88 (94.6%)	107 (93.0%)
MTB INH-resistant	1 (1.0%)	4 (3.5%)
MTB rifampicin-resistant	1 (1.0%)	1 (0.9%)
MTB multidrug-resistant	3 (2.9%)	3 (2.6%)

Acquired *M. tuberculosis* drug resistance was present in 1 of 5 HIV-positive and 6 of 8 HIV-negative patients. White male homosexuals ($N = 8$) accounted for 2 of 3 multidrug-resistant HIV-positive cases and 3 of 6 atypical mycobacterial infections.

In the Western Cape, therefore, HIV infection is highly prevalent in hospitalised patients with tuberculosis. In the black and coloured populations, HIV infection was *not* associated with an increased prevalence of (multi-) drug-resistant tuberculosis. This is in keeping with recent surveys in Malawi and Cote d'Ivoire, which found TB drug resistance to be unrelated to HIV status.[3,4] Our data support the current practice, which is to treat tuberculosis with a standard drug regimen, regardless of HIV status.

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Our peripheral hospitals — crisis or opportunity?

To the Editor: We are in dire need of a vision for our future. The crisis in our peripheral hospitals must not be allowed to translate into failure of our health service.

We need a system that will consolidate the individual departments at each hospital. This includes an adequate number of full-time consultants, who will co-ordinate in-house teaching and CME for MOs, interns and nursing staff, evaluate effectiveness of casualties, wards and OPDs, and run programmes to maintain and improve standards generally.

This high profile of our senior staff will help boost staff morale and stop that sinking feeling!

To achieve this, we need a commitment from the health authorities to provide the posts, and from our universities to provide specialists trained to fulfil this unique role. We need imaginative restructuring of medical education to provide doctors to suit our country's needs at this stage of our history, rather than to provide other Western countries with a constant supply of South African doctors. Universities should perhaps be rewarded by a pro rata subsidy for every graduate who stays on in South Africa, on a year-to-year basis; or should be persuaded to rotate a small proportion of their senior registrars or junior consultants through peripheral hospitals via a roster to give these hospitals some extra consultant cover.

There are many departments of community health around the country that would be only too willing to tackle this challenge. Why are they not being used?

We need dialogue. We need an exchange of ideas. We need lateral thinking. This is our window of opportunity — let us make use of it.

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To the Editor: 'Where there is no vision, the people perish.' How tragically these words ring true when one works in a large, understaffed regional hospital desperately trying to meet the challenge of providing a full regional service.

We applaud the commitment of the government and provincial health authorities to make improved health services available to all South Africans, and acknowledge many of the steps taken towards this. However, I believe that they are failing to see one vital fact; that in order to create strong regional specialist units, we must attract senior clinicians with vision for the department, the hospital and the region. Attract strong heads of department, who are willing to commit themelves for several years, and good juniors will arrive. Build up regional hospital departments, and district hospitals will be supported.

With regard to the government's proposals regarding longer training requirements for South African doctors and de-recognition of British medical training for registration in this country, we must realise that we will never build solid foundations for our hospitals using junior doctors who are here temporarily and by force. Who is to support and teach them?

I work in a regional paediatrics department which from 1994 to 1996 had no leadership whatsoever. Doctors changed by the month, there was very little after-hours cover, and only one of us had any paediatric qualification — a DCH (not mine!). Through word of mouth, and personal contacts overseas, we have in 1 year grown to a department of three doctors with an MRCP (Paed) (UK), three with a DCH (SA), one with part 1 of the MRCP, and a further two with a long-term interest in paediatrics. I believe that, largely due to our energetic leader, our service has improved significantly. But, barring two of us, we are all from the UK! The other two are also not South African. What are we going to do in a few months when most of this team has left to continue structured careers in the UK? Who will take over the challenge and the vision? In 2 years we managed to get only one South African specialist to visit and advise us.

To those who make decisions — we desperately need conditions of service that will draw experienced local clinicians with a long-term vision to the regional services. For surely, while we have no vision, our people are indeed perishing.

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Medical Council's proposed new categories of registration

To the Editor: Thank you for the editorial in the April edition of the *SAMJ.*[1] As a concerned medical practitioner working in a rural hospital I would like clarification from the Medical Council on the current moratorium on the registration of foreign doctors and the proposed new registration of medical practitioners in this country. Having studied proposed new registration categories, I am very concerned about how we are going to staff our hospital in the future.

Mosvold Hospital is a 250-bed hospital situated in deep rural KwaZulu-Natal. It serves a population of 110 000 people who live in scattered homesteads and eke out a living from subsistence farming, which is supplemented by income generated by migrant labourers and pension payouts.

The hospital (currently staffed by 6 medical officers) has an active primary health care service and supervises 5 residential clinics, a mobile clinic service, an under-5 mother and child service and 138 community health workers who each take responsibility for approximately 100 homesteads. Doctors visit the clinics on a weekly basis to see problem patients and to provide CME to the nursing staff.

Between January 1990 and May 1992 there were five medical officers at the hospital. Three of these doctors were South African and the other two were British. Since May 1992 we have been unable to attract *any* South African doctors to Mosvold Hospital, despite a number of adverts in the *SAMJ.* The hospital has been kept going by short-term visits from British doctors who stay anywhere from 3 months to 18 months. Currently there are seven doctors at the hospital — two South Africans and the other five from

THE DANGERS OF PROCEDURE CODING

The transition to more comprehensive clinical recording is one of the many changes that South African healthcare organisations are having to make. The advent of managed care and technological changes in patient treatment demand a more accurate and sophisticated understanding of the healthcare business. In the days of fee for services, managers were principally concerned with revenue management and the type of patient treated was not as important as the consumables that they utilised. As healthcare contracting shifts to per diem and cost per case, managers now have to transition to expense management and must invoke a higher understanding of the actual business.

Refined and accurate clinical coding is central to knowing one's business. Procedure coding, which is all that's needed for fee for service billing, is inadequate for per diem or per case billing or for management information. Funders and providers, with few exceptions, have over-emphasised the new CPT procedure coding system and ignore, at their peril, the need for **both** diagnostic as well as procedure classifications to be able to understand their patient profiles.

The table illustrates how these two codes can be powerful in combination but potentially dangerous in isolation. Providers or funders basing their long term exposure on procedures or diagnoses alone face the risk of over-simplifying the enormous effects of complications. Providers, being the most at risk under fee per case reimbursement, can face serious financial pressure.

Funders, on the other hand, cannot work with undefined margins of error and need equally to understand how patient profiles vary between plans, regions and facilities.

Healthcare managers have basically had an easy job. The survivors will be those that adjust to the realisation that more data and more information are required for their business, not just more services rendered. The introduction of managed care into the South African healthcare arena does not mean that a "win/win" environment cannot be achieved. However, unless there is an understanding of the true picture of business being generated, decisions will be made seeing only a part of the picture.

Providers and payers must work together in order to achieve the most efficient and effective healthcare delivery system. Value Health Services offers a series of non-partisan services and products aimed at implementing and utilising the new coding systems scientifically and to the benefit of health care in South Africa in general.

VALUE HEALTH SERVICES

Clinical Condition	Ave Length of Stay	Ave Cost per Case (Cdn $)	Variance
Bronchitis			
with complication/comorbidity	7.3	$ 4 380	
no complication/comorbidity	4.1	$ 2 979	47%
Major intestinal/rectal procedure			
with complication/comorbidity	12.7	$ 11 583	
no complication/comorbidity	9.3	$ 7 795	49%
Laparotomy			
with complication/comorbidity	8.9	$ 7 991	
no complication/comorbidity	6.5	$ 5 286	51%
Hip replacement			
with complication/comorbidity	11.8	$ 16 723	
no complication/comorbidity	9.4	$ 14 293	17%
Urinary neoplasm			
with complication/comorbidity	7.6	$ 5 169	
no complication/comorbidity	3.3	$ 2 264	128%

Britain; without the support that we get from these British doctors, we would be unable to continue to run the hospital.

The current proposed registration makes it compulsory for every doctor who has not qualified in South Africa to write an examination before being able to practise in this country. While I agree with the sentiment of the document and agree that it is essential that the Council ensure that doctors practising in this country are competent to do so, I feel that this proposed registration may well end up forcing hospitals such as Mosvold to close because we are no longer able to recruit medical staff to assist with the running of the hospital. The (mainly) junior staff that we have had from the UK have been competent and have quickly adapted to local conditions. Many of them would not have come to South Africa if they had been compelled to write an entrance examination.

I would urge those responsible for drafting such recommendations to consider carefully the implications of these recommendations for institutions such as Mosvold Hospital. Furthermore, should these recommendations be accepted, Council should be willing to take responsibility upon themselves to help find suitable doctors for institutions such as Mosvold Hospital.

A J Ross
Acting Medical Superintendent

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DOTS — are we over-optimistic?

To the Editor: An article in a recent issue of *the SAMJ*[1] reports the World Health Organisation's claim that 'as the result of its DOTS (Directly Observed Treatment Short-course) the global TB epidemic is levelling off for the first time in several decades'. In addition, in the press release to which this article presumably refers,[2] Dr Hiroshi Nakajima, Director-General of the WHO, states: 'DOTS is the biggest health breakthrough of this decade in terms of lives we will be able to save', and Dr Kochi, Director of the WHO Global TB Programme says: 'This is the single most important development in the fight against humanity's oldest and most deadly infectious disease since Robert Koch discovered the TB bacillus in 1882'.

These are strong statements, which one would assume are informed by firm evidence. Surprisingly, this is not the case. We have just completed the first edition of a systematic review of trials that examined specific strategies to promote patient adherence to tuberculosis treatment.[3] We included trials that were randomised or pseudo-randomised, tested interventions aimed at improving adherence to curative or preventive therapy for tuberculosis, and contained at least one outcome measure of adherence. We used a comprehensive search strategy and five studies met the prespecified inclusion criteria. Several interventions, e.g. reminder letters to clinic defaulters, assistance of patients by lay health workers, monetary incentives and increased supervision of clinic staff, seemed to be of benefit. None of the completed trials examined DOTS, although we identified two trials in progress that are randomising the specific

strategy of observing ingestion of medication. Results of these studies will be included in future editions of the review as they become available.

The enthusiasm for DOTS is based on the results of non-randomised, often poorly controlled research. We are concerned that selection biases inherent in these studies may have led to over-optimistic estimates of the effect of DOTS. In addition, as we have mentioned elsewhere, co-interventions could potentially explain the supposed benefits of DOTS.[4] DOTS is a specific *strategy* whereby the patient swallows medication under the direct supervision of a third person. DOTS *programmes*, on the other hand, usually consist of substantial effort and investment to improve tuberculosis services, such as better service access, improved drug supplies, patient incentives, defaulter tracing and outreach activities. These parallel interventions, rather than DOTS *per se*, may account for the beneficial effects being attributed to DOTS. The risk associated with the rather simplistic statements from the WHO is that countries may alter their current administrative systems for managing tuberculosis to DOTS without the accompanying parallel interventions and investment. It is therefore our opinion that the recent WHO claims are premature and potentially counterproductive. Carefully designed randomised trials will help provide a more informed policy.

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Bestuurde gesondheidsorg — wie gaan die wenners wees?

Aan die Redakteur: Die menings wat dr. Krüger[1] oor die invloed van bestuurde gesondheidsorg en die kwessie van uitkontraktering en professionele fooie onlangs uitgespreek het, is tiperend van die verlore stryd wat die privaatpraktiserende geneesheer in Suid-Afrika die afgelope 3 dekades voer. Sy menings en voorstelle is niks nuut nie. Inteendeel, ek het al meer as 13 jaar gelede dieselfde argumente gevoer en voorstelle gemaak[2,3] wat geen reaksie van enige kollega of organisasie uitgelok het nie.

Geneeshere kon nog nooit as 'n verenigde front saamstaan nie en noudat die stryd teen die geldbeherende groot besighede verloor is, bly net die vertroeteling van 'n edel geneesheer-pasiënt verhouding en mediese versorging waarop die professie trots is en waarvoor die pasiënt dankbaar is, oor. Die dae van hoë finansiële vergoeding en leefwyse in 'n land wat wemel van armoede is heel waarskynlik verby en ons sal ons maar moet staal om as werknemers van privaat gesondheidsorgfinansierders of die owerhede ons professie te beoefen. Die gans wat vir die afgelope 30 jaar die goue eiers getrou gelê het, is oud en afgeleef en word vervang deur 'n roofvoël wat die kuns het om sy verswakte prooi met dodelike akkuraatheid af te maai.

Miskien is die nasionale gesondheidsversekeringsplan van die regering tog nie so 'n slegte plan nie. Net jammer die privaatpraktiserende geneeshere het nie die geleentheid benut om 'n leidende rol daarin te speel nie. Dit kon 'n deurslaggewende faktor vir die toekoms gewees het!

Robert J E Erasmus

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Gezina
0031

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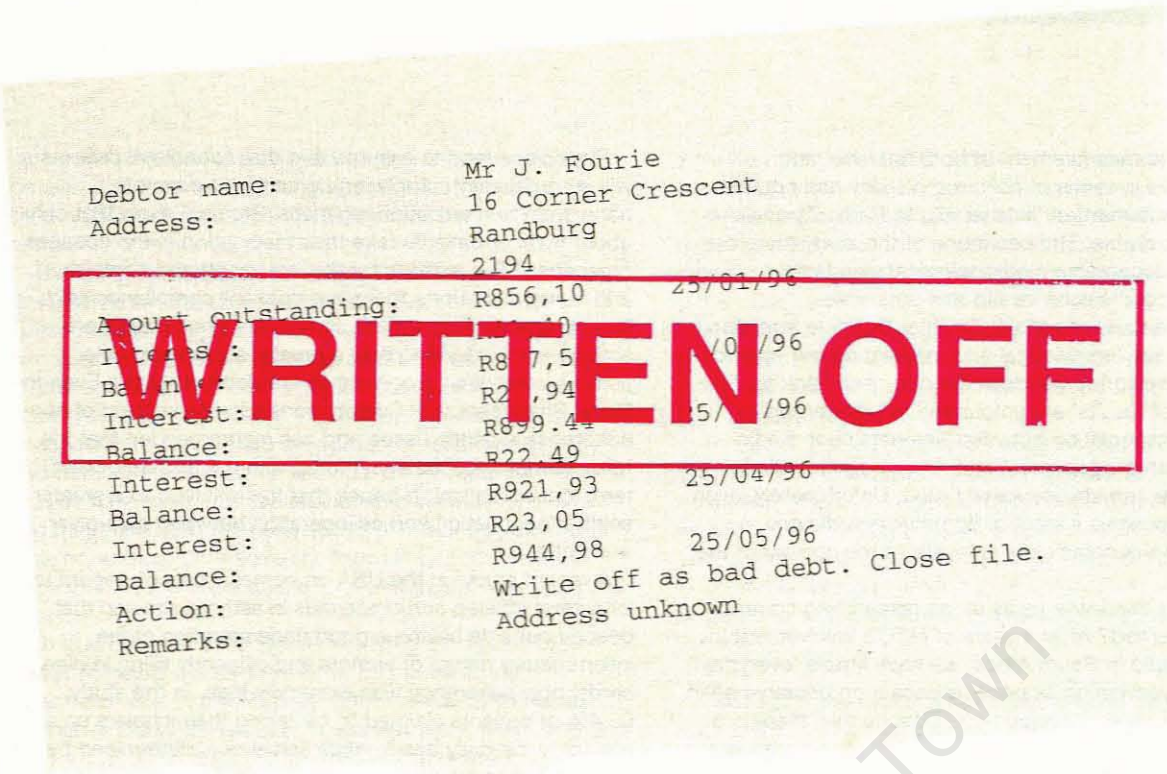
Evaluation of community radio as a medium for health messages

To the Editor: Community radio is a newly developing sector. Very little empirical audience-based research assessing its impact has been conducted. However, more and more organisations are giving this attention. The National Progressive Primary Health Care Network's Media and Training Centre (MTC), together with Radio Zibonele, have started a multifaceted research programme combining quantitative, qualitative and participatory methodology. We therefore took special interest in the letter by Bardwell *et al.*[1] The following comments are based on careful scrutiny of the full research report, in conjunction with the abbreviated version published.

The study was conducted by four undergraduate medical students as part of their Community Health module. It was conducted within a praxis framework, meaning that it was conducted congruently with the values of the sector and that results are fed back in a way that contributes constructively to sector building, and deserves support and special mention on these grounds. However, the project lacked sound methodological guidance. We have several points of critique, given in summary here (a full document is available).

The project is characterised by an over-generalised title, inadequately reflecting the research question. (If you take the title at face value, you have to concede that the research design renders the study internally invalid.) Initially the study is introduced as 'a survey'. Yet, if it claims to be a survey, no claims of either internal or external validity can be made. Later on it becomes clear that a case control design was implemented — the easier design option in the context of the complexities of the research question. The consequences of the lack of control over all variables (especially extraneous variables) inherent in this design, have not been taken into account in either techniques used or the interpretation and presentation of findings, resulting in uncritical over-interpretation. The most fundamental point of critique, however, relates to an inadequate measuring



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instrument: the measurement of both 'attitude' and 'practices' fails in terms of construct validity and content validity; measurement of 'listenership to Radio Zibonele' is crude and unreliable. The backbone of the study therefore falls short of acceptable methodological standards, rendering all conclusions invalid and unreliable.

The only measure of association that warrants attention is the one between 'knowledge' and 'presenting with gastro'. The other 'noteworthy trends in the data' and 'striking consistency of results' are unfounded. All references to measures of association between 'listenership to Radio Zibonele health programmes' and 'knowledge', 'attitude' and 'practices' remain unsubstantiated. Unfortunately, even the reported positive impact of community radio on knowledge of the community is invalid in the context of the study.

Where does this leave us as far as researching community radio is concerned? After 4 years of MTC's involvement in community radio in South Africa, we have ample 'everyday' experiences confirming its positive impact on primary health care and community development. Despite this, there is a strong need to conduct research as a formalised, systematic process that investigates these everyday experiences in an objective, reliable and valid manner. The mere commitment to conducting research (within a praxis framework) is not enough, however; it needs to be backed up by quality output. The whole sector (researchers and users of research — especially community radio stations) is responsible for ensuring that this happens. Researchers need to be held accountable for their methodology and reporting, and users of research need to become informed enough to ask critical questions. For this to happen, research needs to be a demystified, transparent process for everybody — a starting point that mirrors one of the characteristic values in community radio.

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Effective asthma control — time for physician and pharmacist to join forces

To the Editor: We have seen a concerted and successful effort by the National Asthma Education Programme (NAEP) to increase both doctor and patient awareness of asthma. However, the greatest challenge facing asthma care-givers is not lack of patient awareness, but a pervasive non-compliance that occurs universally with inhaled 'preventer' medication.

For optimal asthma control, prolonged and consistent anti-inflammatory therapy is required even in the absence of symptoms. To achieve consistent medication usage, doctors will actively have to enrol the assistance of the community pharmacist. Use of the pharmacist as a medication supervisor and an asthma educator will greatly improve adherence to inhaler medication regimens.

Physicians tend to assume that their 'obedient' patients will automatically comply, and greatly overestimate adherence to medication regimens. Studies[1] show that only about 50% of patients take their medication in the dosages prescribed. The reasons for this are varied and complex, and numerous efforts to improve patient compliance have been disappointing. Unless community pharmacists are actively encouraged to play a greater supervisory role, asthma prevention programmes are doomed to fail. Even the Royal Pharmaceutical Society considers the subject of non-adherence a priority issue and has recommended that the term 'compliance' be substituted with the less autocratic term 'concordance'. It hopes that this will lead to a greater spirit of negotiation and co-operation between care-giver and patient.[2]

A recent study[3] in the USA on compliance with regard to long-term inhaled corticosteroids in asthma showed that despite patients having a good understanding of the inflammatory nature of asthma and diligently filling in diary cards, non-adherence was extremely high. In the study, 95.4% of patients claimed to be taking their inhalers on a regular twice-daily basis, while actual use, determined by an electronic monitor, was only 58.4%. An interesting observation was that the more poorly controlled asthma sufferers who required 'rescue' oral steroids or hospitalisation had even worse compliance data. In this sub-group, only 13.7% took their inhaled corticosteroids regularly as prescribed. This study has far-reaching implications when one considers the financial impact of wasted medication, unnecessary hospitalisation for exacerbations, and worsening long-term morbidity due to uncontrolled illness.

It is imperative that physicians and pharmacists bury old animosities and work together as 'educators', so as to increase the awareness of non-compliance and find ways of combating it via mutual co-operation.

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Migrancy and HIV/STDs in South Africa — a rural perspective

To the Editor: Williams and Campbell[1] raise the important issue of the role of migration in the spread of HIV/STDs in their discussion of a proposed intervention around a mining centre near Johannesburg. Recognising that an intervention aimed solely at mineworkers would be futile, they argue that interventions must be targeted not only at mineworkers 'but also at members of the broader communities surrounding the goldmines, in which mineworkers live out their social and sexual lives while they are away from home'.

This represents an important strategy, but could go further. The vast majority of mineworkers are migrant

labourers from rural areas. Interventions must therefore also be aimed at rural partners of migrants who themselves may be at increased risk for HIV/STD infection because their partners are migrants.

The link between migration and HIV/STD has been documented in the few studies that have been done.[2-6] These studies suggest that 'migration of poor, rural and young sexually active individuals to urban areas . . . played a prominent role in the dissemination of HIV globally'.[4] In South Africa, the system of migrant labour has been shown anecdotally to exacerbate the spread of tuberculosis,[7] HIV[8] and other STDs.[9] However, with few exceptions, all these studies have examined the relationship between HIV/STD and migration from the perspective of *urban* migrants at their workplace. Consequently, they fail to document the impact of — and implications for — migrants' return to rural areas.

To that end, we are currently studying the social and epidemiological consequences of migration at the household level in the Hlabisa district of KwaZulu-Natal. Formative research has been underway since May 1996 and is aimed at understanding the patterns of migration into and out of the district, knowledge and awareness of HIV/STDs, local health beliefs and health-seeking behaviours, and patterns of sexual networking. A variety of qualitative research methods are being used, including the establishment of more than 20 key-informant households which are serving as long-term case studies, ongoing work with school students who are keeping daily logs of the composition of their households, observations at local taxi ranks, and interviews with traditional healers. The second phase of the research, due to begin in June 1997, will be a cross-sectional study aimed at testing the hypothesis that migrants and their rural partners are at increased risk for contracting HIV/STDs compared with non-migrants and their partners.

Our preliminary research suggests that nearly two-thirds of households in the district include a male who is a migrant. While nearly one-third of male migrants from Hlabisa work in the Johannesburg area, 48% work in towns scattered along the northern Natal coastline (within a 2-hour drive of Hlabisa) and 18% are in Durban (a 3½-hour drive from Hlabisa). These findings challenge the stereotype that migration is generally long-distance and long-term.

Our research also challenges the commonly held assumption that it is only men who migrate. While we have not yet measured the prevalence of migration among women, we do know that women who migrate tend to stay much closer to home than their male counterparts do. Of the women migrants we have identified, none works in Johannesburg and the majority work within a 1 - 2-hour drive from Hlabisa.

Patterns of migration in South Africa did not simply arise by chance. On the contrary, a myriad of laws under apartheid prohibited black South Africans from settling permanently in 'whites-only' areas. Migration patterns in South Africa, although incompletely documented, tend to be 'oscillatory' or circular, with migrant men and women maintaining close links to their rural homesteads. These patterns, however, are clearly in flux and these changes may bring with them important implications for the spread — and control — of HIV-STDs. In the past, for example, migrants who went from Hlabisa to work in Johannesburg could return home only once a year. Now, however, with the lifting of restrictive laws, improvements in the transportation systems and more flexible work contracts, men from Hlabisa working in Johannesburg are able to return home much more often — on average about once every 2 - 3 months. One impact of this change in migration patterns may well be that rural partners are more frequently exposed to potential infections, and are therefore more likely to be infected with HIV and other STDs.

The work that Williams and Campbell and their colleagues propose around the goldmines is important indeed. At the same time, there is a need to understand migration from both ends of the spectrum. Appropriate HIV/STD treatment and prevention programmes must be targeted to migrants — both at their place of work and at home. They must also be targeted to rural partners of migrants if the epidemic is to be curtailed.

Funding for this research has been provided by the South African Medical Research Council and the Fogarty International Centre, NIH.

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Sarafina III?

To the Editor: As an intern, I would like to share some of my objections and those of my colleagues to the proposed plan of community service. When we embarked on a career in medicine we did not enter into any contract with the Department of Health or Dr Zuma with regard to our conscription.

It enrages us that we have neither been consulted nor given the opportunity to voice our opinions. Is it not arrogant and misinformed to presume that individuals enter the medical fraternity purely for altruistic reasons?

Another of the many unaddressed issues is why one profession is being targeted. Why not force accountants to

do government audits, lawyers to provide legal aid, and engineers to develop our rural infrastructure?

The transparency of the pseudonym 'vocational training' has been revealed, and it is obvious that it is the antithesis of an incentive-driven voluntary scheme. To justify this, suddenly our competence and that of our teachers (many of them internationally recognised in their fields) are being questioned. Why, then, are we considered competent for employment in First-World countries?

The Department of Health's shortsightedness is borne out by the following unaddressed issues: (*i*) how can supervisory posts be created overnight in satellite hospitals when academic institutions are reeling from the current critical shortage of teaching staff?; and (*ii*) why not make family practice a specialty rather than force demotivated doctors into areas of medicine which hold no interest for them?

In this new era of democracy and constitutional rights, I find it highly contradictory and, ironically, reminiscent of the previous regime. The vocational training scheme is violation of an individual's right to equality, right against forced labour, and right to choose one's place of residence and one's profession.

Leaders in this country should be held accountable for their decisions. Young doctors would willingly stay and serve their country, but it would seem that Dr Zuma plans to drive all doctors from South Africa, in addition to eradicating measles and polio.

Marc Coughlan

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Abortion objectors — rights and responsibilities

To the Editor: The Choice of Termination of Pregnancy Act passed recently in Parliament has brought with it some major logistic problems, particularly as a result of doctors and nurses at many hospitals having moral objections to being involved. These objections range from not wanting to have anything to do with women requesting abortion to agreeing to all but the handling of the manual vacuum aspirator themselves. Many, if not most, objections are based on reluctance to take or participate in the taking of an innocent baby's life. Often, however, zeal for the cause may result in avoidance of careful history-taking, failure to do a full and dignified examination, and sloppy handling of the patient. This is inexcusable and may be medicolegally negligent. I have on one occasion found that a woman requesting an abortion was not pregnant, and another had an ectopic gestation. I do not object to confirming the presence of a normal pregnancy and declaring the woman medically fit, even if she would have been assessed at any antenatal booking clinic by a qualified midwife had she wished to keep her pregnancy. Preliminary evaluation of these women should really be performed at an antenatal clinic.

To avoid the problems of directive counselling it is probably a good idea for the waiting rooms of gynaecological OPDs to have clearly and simply explained options and alternatives in the appropriate languages for women before they are seen and examined by staff. They may then be able to enquire further in an informed manner. The practitioner who objects has every right to refuse to write up or administer misoprostol to induce an abortion and it is prudent for medical superintendents to organise presigned scripts or willing practitioners to oversee its use.

I think it is indefensible for a doctor or nurse with objections to refuse to see a patient arriving at hospital with a threatened, inevitable or incomplete abortion, even in the knowledge that an abortion had been procured. Certainly once the baby has been delivered, retained products constitute a grave risk to the mother's future health and fertility. Refusal to attend to her would surely be medicolegally culpable. If the objectors feel that the method by which she aborted constitutes grounds for refusal to treat, then the corollary exists that they must refuse to see injured drunk drivers or attempted suicides. However, agreement to see these patients on an emergency basis ensures that what would previously have been regarded as an 'emergency' has now become 'routine' work and thus forces staff who object strongly to participate in abortion work to keep the system running. This is stressful and highly unsatisfactory for the staff and should motivate administrators to designate separate staff and facilities for fetocidal purposes.

Some doctors and nurses attempt to stigmatise and even persecute colleagues who are prepared to do abortions and run the risk of allowing their differences of opinion to spill over into interpersonal relationships, which affects co-operation on other work. This only polarises the profession and generates more heat than light. The fact of the matter is that abortionists and objectors exist, are employed in the same institutions and will not resign their jobs. A clear statement of one's moral position does not mean a call to arms. The need to co-operate fully to provide the vast remainder of O&G responsibilities is critical and should not be threatened by this divisive issue. To expect one willing member to fulfil routine duties plus deal with the abortion load is grossly unfair and may be construed as mere avoidance of work.

With the passing of the new legislation, rights have been afforded many women without the means to accede to them. Little if any attempt was made to assess the willingness of current medical staff to fulfil the requirements of the new Act. If the current impasse is not broken by means of creative co-operation, the stalemate may threaten the very survival of our fragile medical network to the eventual detriment of all.

If the government does not take the issue of conscientious objection seriously, we face a very bleak and desperate future.

Harvey Ward

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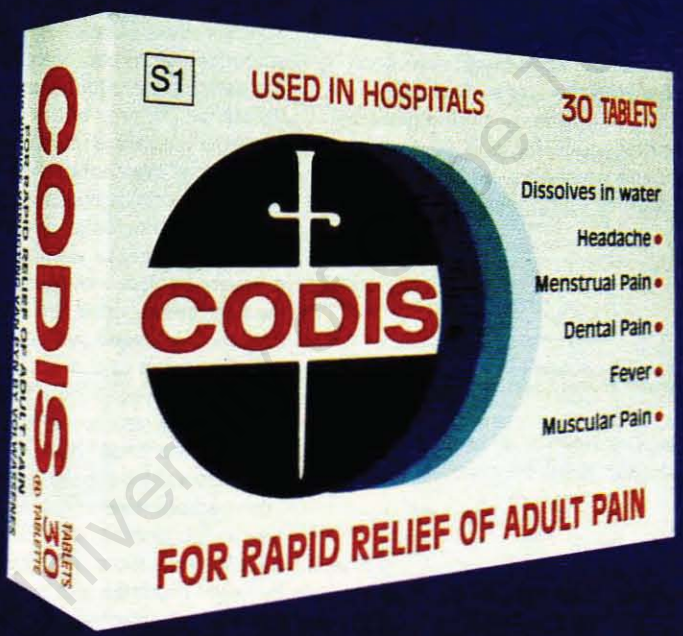
Pinelands, W Cape

Diagnostic disagreement with malaria diagnosis in Mpumalanga

To the Editor: Durrheim *et al.*[1] describe disagreement following the examination of blood smears for malaria. The specimens were examined successively at four different laboratories. Experience has shown that repeated

Here is your first lesson in pain relief



There is no second lesson

You need look no further to give quick and effective pain relief to your patients. Codis, due to its soluble nature deals its fast acting ingredients to create effective blood levels and ensuring that rapid onset of action.

It's no real surprise then that Codis is used extensively *in most South African hospitals.* Making it the preferred analgesic for headaches, muscular pain, toothache, dysmenhorrhea and any other inflammatory injury.

Isn't it time you taught pain a lesson?

examination and cleaning result in loss of material and fading of the stain. Specimens received at one of the laboratories were in this category. When parasitaemia is at a low level, detection may be problematic and may only take place at a subsequent examination. No information on parasite levels was given. A further confounding factor is the differing quality and condition of microscopes in use. The laboratory results given are unsatisfactory and require further investigation, especially those of laboratory 2 when compared with the other three.

A quality assurance service has been operational for several years in the Northern Province involving the confirmation of positives and re-examination of at least 10% of negatives undertaken at a separate laboratory. Apart from a few instances where faulty equipment or microscopist stress has been responsible, 99.4% of results have been confirmed during the past 2 years. Training involves an initial course using a defined syllabus, regular refresher courses for all microscopists and recall for further training of those microscopists who give discrepant reports.

To ensure uniformity when laboratories are compared, it would be preferable to make multiple specimens from each patient or for specimens to be protected by sealed cover glasses. Parasite counts should be made by all participating laboratories. Confirmation of diagnosis with a PCR/ molecular-based test for parasite presence and species should be undertaken and used for comparison.

C F Hansford
C M van Vuuren

Malaria Co-ordinating Centre
Tzaneen, Northern Province

1. Durrheim DN, Becker PJ, Brink A. Diagnostic disagreement — the lessons learnt from malaria diagnosis in Mpumalanga. *S Afr Med J* 1997; **87:** 609-611.

(A response to this letter by Durrheim *et al*. arrived too late for inclusion in this issue of *SAMJ*. It will be published in August.)

Impact of antimicrobial resistance and antibiotic choice on the outcome of pneumococcal meningitis

To the Editor: I noted with interest the paper on the epidemiology of post-neonatal bacterial meningitis in Cape Town children.[1] An important observation of the study, highlighted in the text and the abstract, is the poor outcome of pneumococcal meningitis relative to meningococcal or haemophilus meningitis. Of the 10 deaths in the study, all occurred among children with pneumococcal meningitis. The case fatality rate of 38% (10/26) is not unusual in series of patients with pneumococcal meningitis. For example, the case fatality rate among 84 children with pneumococcal meningitis at Baragwanath Hospital from 1989 to 1991 was 32%.[2] While the authors identified young age as a risk factor for severe disease or death among children with haemophilus and meningococcal disease, they were unable to find such an association for pneumococcal meningitis. In our study at Baragwanath Hospital,[2] the outcome of pneumococcal meningitis was poor, since relatively penicillin-resistant strains were common and treatment commenced with penicillin and/or chloramphenicol. Hussey *et al*.[1] give no data on the susceptibility of the pneumococci they isolated, and no data are presented on the antibacterial agents used to treat meningitis during their study. It would be interesting to know whether the excess mortality from pneumococcal meningitis they reported was due at least in part to the presence of resistant strains, and whether this mortality could be reduced by the use of third-generation cephalosporins such as cefotaxime or ceftriaxone for empirical management.

K P Klugman

MRC Pneumococcal Diseases Research Unit
South African Institute for Medical Research and
University of the Witwatersrand
Johannesburg

1. Hussey G, Schaaf H, Hanslo D, *et al*. Epidemiology of post-neonatal bacterial meningitis in Cape Town children. *S Afr Med J* 1997; **87:** 51-56.
2. Friedland IR, Klugman KP. Failure of chloramphenicol therapy in penicillin-resistant pneumococcal meningitis. *Lancet* 1992; **339:** 405-408.

Drs Hussey and Hanslo reply: We thank Professor Klugman for drawing attention to the association between a poor outcome of pneumococcal meningitis and occurrence of penicillin-resistant strains. Our study focused on the epidemiology of post-neonatal bacterial meningitis, and was not designed to address antimicrobial therapy or the resistance patterns of the causative organisms. The limited available data were used to determine the presence of any risk factors. We cannot therefore confirm an association between mortality and penicillin resistance. During the study period the empirical antimicrobial treatment of presumed bacterial meningitis was a combination of penicillin and chloramphenicol. However, third-generation cephalosporins were frequently used, especially in situations where pneumococci were seen on Gram stains of cerebrospinal fluid.

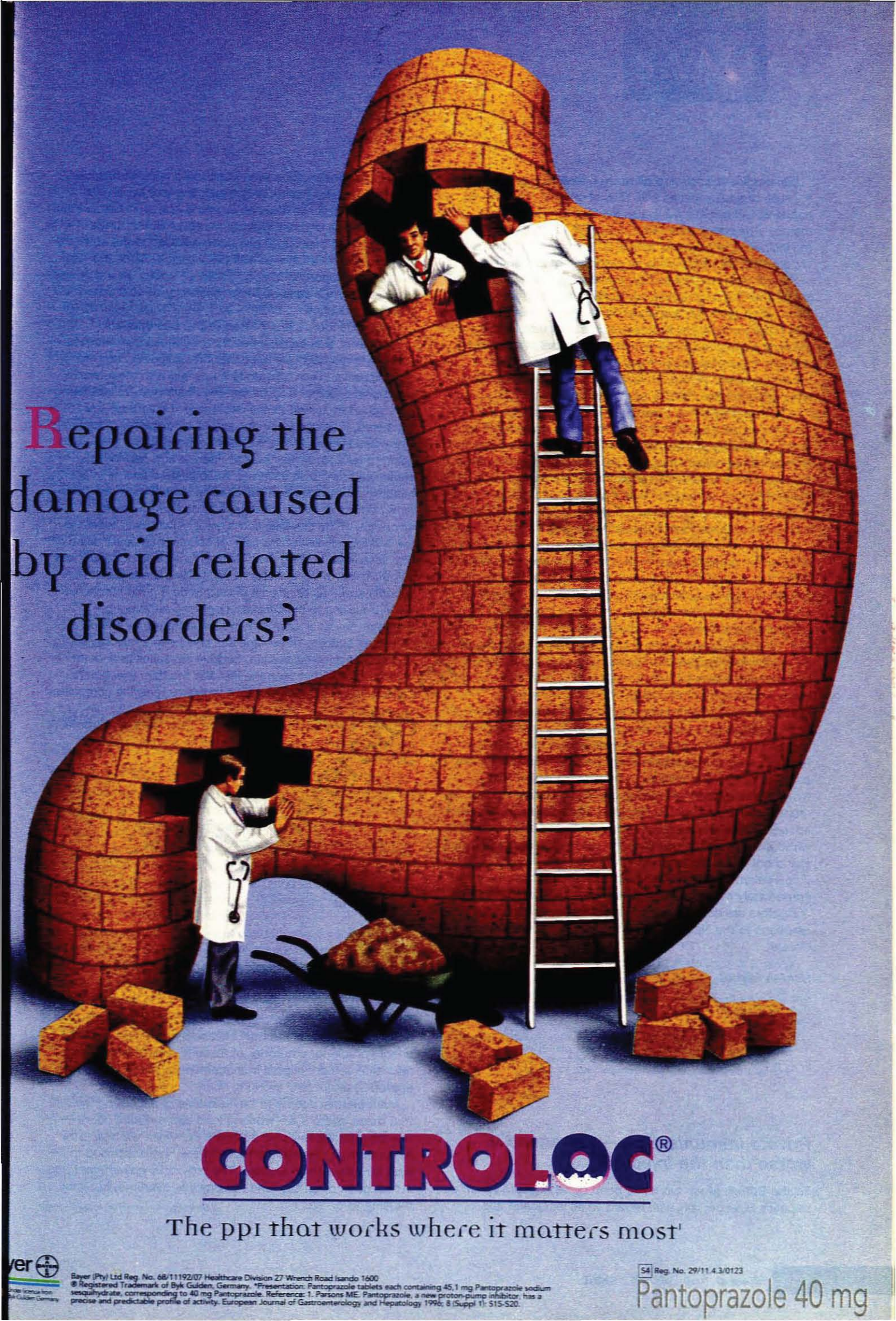
Subsequent to this epidemiological study, a further one was undertaken to investigate the antimicrobial resistance patterns of pneumococcal isolates (G Hussey — unpublished data). Three of 26 strains from CSF (11.5%) were found to be resistant to penicillin (two with intermediate resistance and one fully resistant). The mortality rate in this cohort of children was 27%. The number of patients was too small to determine any clear association between mortality and penicillin resistance. The rate of penicillin resistance in blood culture isolates was 24/88 (27.3%), with 22 of these showing intermediate resistance.

In the light of the emerging resistance of pneumococci to penicillin and the reported association between mortality and penicillin resistance, our current antibiotic policy for the treatment of pneumococcal meningitis is a third-generation cephalosporin.

Lamotrigine and serious skin reactions

To the Editor: The Medicines Control Council has been alerted to the potential for serious skin reactions with the use of lamotrigine. Paediatric patients are at special risk.

Lamotrigine is an anti-epileptic drug indicated as monotherapy or add-on treatment for partial epilepsy, with or without secondary generalised tonic-clonic seizures, and in primary generalised tonic-clonic seizures in adults. Lamotrigine should be used in children aged 2 - 12 years as add-on treatment for partial epilepsy (with or without secondary generalised tonic-clonic seizures) that is not satisfactorily controlled with other anti-epileptic medicines.



Lamotrigine is known to cause skin rashes in approximately 5 - 10% of adult patients and 17% of children. This usually happens, but not always, within the first 6 - 8 weeks of therapy. The concomitant use of sodium valproate increases the incidence of skin reactions to 21% in adults and 34% in children (Glaxo-Wellcome — unpublished data, 1994).

Serious skin reactions, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, occur in 1 in every 1 000 adult patients, and in approximately 1 in 300 to 1 in 100 children.[1] In addition to age, the risk of rash is also strongly associated with: (*i*) high initial doses of lamotrigine, which exceed the recommended dose escalation of lamotrigine; and (*ii*) concomitant use of sodium valproate, which doubles the half-life of lamotrigine.[2]

A further concern is a hypersensitivity reaction to lamotrigine associated with variable symptoms, including fever, lymphadenopathy, facial oedema, haematological and hepatic abnormalities that may progress to disseminated intravascular coagulation and multi-organ failure. This syndrome may or may not be associated with a rash.[1]

Based on this information, the following recommendations have been included in the product labelling:

1. Lamotrigine remains indicated as add-on treatment in children for partial epilepsy not adequately controlled with other anti-epileptic medicines. The safety and efficacy of lamotrigine as monotherapy in children under 12 years of age have not been established, and there are serious misgivings about safety in this age group.
2. The dosage instructions in the package insert should be strictly followed, especially when used in combination therapy with valproate where one-tenth to one-fifth of the normal dose is recommended. The initial dose and subsequent dose escalation should not be exceeded.
3. In children, the initial presentation of a rash may erroneously be attributed to the usual childhood diseases. Physicians should exercise a high index of suspicion in all patients (adults and children) who present with a rash, lymphadenopathy and/or fever, especially during the first 3 months of therapy with lamotrigine. Patients developing this adverse effect should be promptly evaluated and lamotrigine withdrawn immediately unless the symptoms are clearly found not to be drug-related.
4. Patients should be advised to see their doctor immediately if they develop a skin rash or fever.

Doctors are encouraged to report these adverse drug reactions to the National Adverse Drug Event Monitoring Centre.

Ushma Mehta

National Adverse Drug Event Monitoring Centre
Medicines Control Council
Cape Town

1. Glaxo Wellcome. A risk-benefit assessment of lamotrigine (Lamictal) and serious skin reaction in children, 24 March 1997 (In-house publication).
2. Yuen AWC, Land G, Weatherly BC, Peck AW. Sodium valproate acutely inhibits lamotrigine metabolism. *Br J Clin Pharmacol* 1992; **33:** 511-513.

Private immunisation — the agony is worse than the injection

To the Editor: Much work has been done in South Africa on hepatitis B, especially with regard to its incidence and relation to cancer of the liver. Although a vaccine has been available for a long time, only health workers have been routinely immunised. The carrier rate is about 14.5% in rural KwaZulu-Natal and half that in the rest of South Africa.

Since 1995, hepatitis B has been included in the statutory immunisation of infants at well-baby clinics. Since this has left a large proportion of schoolchildren unimmunised, it was felt that a private scheme for immunising schoolchildren could be launched.

What a Pandora's box of trouble this has opened!

Resistance was experienced from local health authorities, who felt that it might be unethical; this ultimately required state approval. Then a complaint was laid with the Interim Medical and Dental Council that the Pharmacy Act was being violated (even though all the doctors on the scheme were registered, 2 as paediatricians and all as dispensing doctors). Many general practitioners on school boards said that it was going to take away their practice (few, if any, had in fact immunised their own children). Headmasters and headmistresses said that the children should go to their own family doctor or health clinic, and all showed woeful ignorance of the disease, and a great naivety with regard to the children at school. Parents, of course, had never heard of the disease and taking their children to a clinic or doctor was therefore out of the question. Even getting the names of schools and their principals proved to be a great hurdle, let alone travelling to the schools, or getting sufficient co-operation to use a room or hand out literature for the parents.

Getting supplies of vaccine has also been a major problem because of price fluctuation. Sudden increases in price of more than 25% between the first and the third injection have caused immense administrative problems, and the companies concerned have a 'take it or leave it' attitude.

Surely, somewhere along the line, there should be more co-operation with regard to what is an advancement of universal immunisation?

It was also painfully obvious that there is still a great need for knowledge and publicity in preventing a potentially fatal disease and for the manufacturers of the vaccine to keep prices down to levels that would make private immunisations within reasonable reach of all.

C R Rainier-Pope

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Rondebosch, 7700

Overtime for hospital doctors in the Eastern Cape

To the Editor: The working conditions of doctors in provincial service in the Port Elizabeth metropole are totally unacceptable for a number of reasons, one of which is the refusal of senior hospital management to adopt a reasonable approach to doctors' working hours.

Management seems to be incapable of accepting the fact that these doctors are working long and arduous hours — well beyond the required 56 hours per week without extra remuneration, despite the fact that they are supposed to be paid for all overtime worked. The provincial government has in fact been given the funds to pay this overtime, but it is refusing to do so.

In July 1996 I was told that overtime worked would be paid for, and I subsequently agreed to work beyond the required 56 hours. In October it became obvious that the overtime worked was not going to be paid. I subsequently withdrew my services for work beyond 56 hours.

I wrote a letter to the superintendent of the hospital concerned and was informed that despite an average of 6.5 hours extra overtime per week over a period of 25 weeks, management had decided that overtime would only be paid after 70 hours worked and so I did not qualify. This decision was based on the wilful misinterpretation of a *Medigram* (August 1996) stating that overtime should be capped at 70 hours.

In consultation with Mr Peter Brewer of MASA it has been decided to pursue this matter further, hopefully to a satisfactory conclusion.

I feel that it is time for the doctors of the Eastern Cape to make a stand for what is right and just, not only in our own interests but in the interests of the patients we serve.

I urge all doctors who have overtime owing to them as from 1 July 1996 to: (*i*) write to their superintendents requesting that they be paid for the overtime owing; (*ii*) attach duty rosters and time sheets to prove their claim; and (*iii*) write to Mr Peter Brewer of the MASA requesting that he attend to the matter if the overtime payment is refused — if they are not members of the MASA they can always join!

J H Jackson

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Theesecombe
Port Elizabeth, E Cape

Dietary management of diabetes mellitus

To the Editor: An extract from an Association for Dietetics in Southern Africa (ADSA) Position Statement on dietary management of diabetes mellitus was used in the Diabetes Mellitus Clinical Guidelines published as part 3 of the April 1997 *SAMJ* (pp. 493-512).

Unfortunately the compilers of the Guidelines did not acknowledge all those who were involved in the compilation of the ADSA Position Statement, namely A Badenhorst (Convenor), J Badham, R Blaauw, A Dannhauser, W du Toit, C Herbert, J Johnson, E Menssink, C Peberdy, N Silvis, M Slabber, P Wilson and R Wilson.

Because the ADSA document used by the compilers of the Guidelines was not the final version, the Guidelines contain two further errors.

Under the heading 'Carbohydrates' (p. 504), the second sentence should read: 'The daily consumption of a diet high in complex carbohydrates and ideally containing *approximately 3 g dietary fibre* (non-starch polysaccharides) per 1 000 kJ, is recommended.'

Under the heading 'Sweeteners' (also p. 504), the final sentence should read: 'More research is needed to identify the effects of long-term use of *non-nutritive sweeteners* in humans, especially pregnant and lactating women.'

The full ADSA Position Statement will be published in a future 'Clinical Nutrition' issue of the *SAMJ*.

Penny Love
Executive Committee: Publications
Association for Dietetics in South Africa

Taylepiece

The Doctour's Tayle

Whan thatte Marche wyth hys shoures pissynge doun
Thatte brynge swich floddes to every parte of toun
Ther cam oon Zuma, a learned doctour of phisik
In al thyss world ne was there noon she lik
She wot the reason for thyss contree's jeopardye
And nameth AIDS the roote cause of thyss maladye
And whatte AIDS engendred, and its discomfiture
She was a verray parfit praktisour
She vowyd thyss dreddfol pestylaunce to cure
For alle thyss contree's populaycioun — eyven Boer.

For God pardee, the cure was wel wythin her kenne
For in her praktyse hadde she met an actynge gentlemanne
To maketh musiekalles was hys wone
For he was Fredde Aystayre's owene sone
Wel koude he dressyth maydens in tyte gymmslyps
Wych hydeth nat theyre rounded bottoums nor theyre hyps.
To Woodstokke hadde he been on pilgrimage
And kenned the virtuye of artystik budgette large
Ngema was thyss actynge manne y-klept
And forteen millyon randes dyd he gretefullie axept.

Thyss musiekalle, eftsoons, was oonlie partlie new —
And twas the reason he y-klept it '*Sarafina two*'.
For tho a musiekalle wyth trobbles is bisette
Thyss forteen millyon kept him cleerly oot of dette.

The learned doctour Zuma natheless hath nat been praysed
And opposicioun membres mayde her soore amayzed
Wyth jalous cryes for lye detectour tests
(Wych sheweth opposicioun spekers are lyke pests).

God wot, the messauge of thyss musiekalle is cleer:
'Forsaketh nakyd screwynge throo-oot the yeyre
And tho ye be a lustfoll and a likerous boy
Tis ofte tymes sayffer to do the toyi-toyi.
And tho ye fynde it gretely combersom
If screw ye must, thanne use oon rubberysed condom!

Douglas Tobias

Durban

This item appeared in the April 1996 Natal Inland Branch newsletter and we thank the Editor, Dr Rod Inglis, for permission to reprint it.

SCHEDULING STATUS: S3
PROPRIETARY NAME AND DOSAGE FORM:

COZAAR* COMP Tablets

COMPOSITION

Each COZAAR COMP tablet contains 50 mg losartan potassium and 12,5 mg hydrochlorothiazide.
COZAAR COMP contains 4,24 mg (0,108 mEq) of potassium.

PHARMACOLOGICAL CLASSIFICATION

A 7.1.3 Other hypotensives

PHARMACOLOGICAL ACTION

COZAAR COMP (losartan potassium-hydrochlorothiazide) combines an angiotensin II receptor (type AT_1) antagonist and a diuretic, hydrochlorothiazide.

Losartan

Angiotensin II, a potent vasoconstrictor, is the primary active hormone of the renin-angiotensin system, and a major determinant of the pathophysiology of hypertension. Angiotensin II binds to the AT_1 receptor found in many tissues (e.g., vascular smooth muscle, adrenal gland, kidneys, and the heart) and elicits several important biological actions, including vasoconstriction and the release of aldosterone. Angiotensin II also stimulates smooth muscle cell proliferation.
Losartan is a synthetic, orally active compound which binds selectively to the AT_1 receptor. Both losartan and its pharmacologically active carboxylic acid metabolite (E-3174) block the actions of angiotensin II, regardless of the source of synthesis.
Losartan binds selectively to the AT_1 receptor and does not bind to or block other hormone receptors or ion channels important in cardiovascular regulation. Furthermore, losartan does not inhibit ACE (kininase II), the enzyme that degrades bradykinin. Consequently, effects not directly related to blocking the AT_1 receptor, such as the potentiation of bradykinin-mediated effects or the generation of oedema are not associated with losartan.

Hydrochlorothiazide

The mechanism of the antihypertensive effect of thiazides is unknown. Thiazides do not usually affect normal blood pressure.
Hydrochlorothiazide is a diuretic and antihypertensive agent. It affects the distal renal tubular mechanism of electrolyte reabsorption. Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. Natriuresis may be accompanied by some loss of potassium, magnesium and bicarbonate.
After oral use diuresis begins within 2 hours, peaks in about 4 hours and lasts about 6 to 12 hours.

Losartan Potassium-Hydrochlorothiazide

Losartan and hydrochlorothiazide are additive in their antihypertensive efficacy.

PHARMACOKINETICS

Losartan

Absorption

Following oral administration, losartan undergoes first-pass metabolism, forming an active carboxylic acid metabolite and other inactive metabolites. The systemic bioavailability of losartan tablets is approximately 33 %. Mean peak concentrations of losartan and its active metabolite are reached in 1 hour and in 3 – 4 hours, respectively. There was no clinically significant effect on the plasma concentration profile of losartan when COZAAR COMP was administered with a standardized meal.

Distribution

Both losartan and its active metabolite are ≥ 99 % bound to plasma proteins, primarily albumin. The volume of distribution of losartan is 34 liters. Studies in rats indicate that losartan crosses the blood-brain barrier poorly, if at all.

Metabolism

About 14 % of intravenously- or orally-administered dose of losartan is converted to its active metabolite.

Elimination

Plasma clearance of losartan and its active metabolite is about 600 ml/min and 50 ml/min, respectively. Renal clearance of losartan and its active metabolite is about 74 ml/min and 26 ml/min, respectively. When losartan potassium is administered orally, about 4 % of the dose is excreted unchanged in the urine, and about 6 % of the dose is excreted in the urine as active metabolite. The pharmacokinetics of losartan and its active metabolite are linear with oral losartan potassium doses up to 200 mg.
Following oral administration, plasma concentrations of losartan and its active metabolite decline polyexponentially with a terminal half-life of about 2 hours and 6 – 9 hours, respectively.
Both biliary and urinary excretion contribute to the elimination of losartan and its metabolites. Following an oral dose of 14C-labelled losartan in man, about 35 % of radioactivity is recovered in the urine and 58 % in the faeces.
Following oral administration in patients with mild to moderate alcoholic cirrhosis of the liver, plasma concentrations of losartan and its active metabolite were, respectively, 5-fold and 1,7-fold greater than those seen in young male volunteers.
Neither losartan nor the metabolite can be removed by haemodialysis.

HYDROCHLOROTHIAZIDE

When plasma levels have been followed for at least 24 hours, the plasma half-life has been observed to vary between 5,6 and 14,8 hours. Hydrochlorothiazide is not metabolized but is eliminated rapidly by the kidney. At least 61 % of the oral dose is eliminated unchanged within 24 hours. Hydrochlorothiazide crosses the placenta but not the blood-brain barrier.

LOSARTAN POTASSIUM-HYDROCHLOROTHIAZIDE

In a pharmacokinetic interaction study, hydrochlorothiazide 12,5 mg did not alter the pharmacokinetics of losartan 50 mg and vice versa.

INDICATIONS

COZAAR COMP is indicated for the treatment of hypertension in patients established on identical doses of the individual agents.

CONTRA-INDICATIONS

COZAAR COMP is contra-indicated in patients who are hypersensitive to any component of this product.
Because of the hydrochlorothiazide component, this product is contra-indicated in patients with anuria or hypersensitivity to other sulfonamide-derived drugs.

Pregnancy

When pregnancy is detected, COZAAR COMP should be discontinued as soon as possible. Not to be used in pregnancy as teratogenicity has been shown in experimental animals.
Thiazides cross the placental barrier and appear in cord blood. The routine use of diuretics in otherwise healthy pregnant women is not recommended and exposes mother and foetus to unnecessary hazard including foetal or neonatal jaundice, thrombocytopaenia and possibly other adverse reactions which have occurred in the adult. Diuretics do not prevent development of toxaemia of pregnancy and there is no satisfactory evidence that they are useful in the treatment of toxaemia.
Women of childbearing age should ensure adequate contraception.

Paediatric Use

Safety and efficacy in children has not been established.

Lactation

DOSAGE AND DIRECTIONS FOR USE

The usual dose of COZAAR COMP is one tablet once daily. For patients who do not respond adequately to COZAAR COMP 50 mg/12,5 mg, the dosage may be increased to two tablets once daily. More than two tablets daily are not recommended. The maximum antihypertensive effect is attained within three weeks after initiation of therapy.
COZAAR COMP should not be initiated in patients who are intravascularly volume-depleted (e.g., those treated with high-dose diuretics).
COZAAR COMP is not recommended for patients with severe renal impairment or for patients with hepatic impairment (see **SPECIAL PRECAUTIONS**).
No initial dosage adjustment is necessary for elderly patients.
COZAAR COMP may be administered with other antihypertensive agents, particularly calcium channel blockers and β-blockers.
COZAAR COMP may be administered with or without food.

*Registered Trademark of E.I. du Pont de Nemours and Company, Wilmington, Delaware, U.S.A.

SIDE EFFECTS AND SPECIAL PRECAUTIONS

Side Effects

In controlled clinical trials for essential hypertension, dizziness and asthenia/fatigue were observed in patients treate with COZAAR COMP.

Losartan potassium

In controlled clinical trials for essential hypertension, dizziness, orthostatic hypotension, headache and rash, were sid effects reported.
Other adverse experiences that have occurred with losartan are:
Body as a Whole: Abdominal pain, asthenia/fatigue, chest pain, oedema/swelling
Cardiovascular: Palpitation, tachycardia
Digestive: Diarrhoea, dyspepsia, nausea
Musculoskeletal: Back pain, muscle cramps
Nervous/Psychiatric: Dizziness, headache, insomnia
Respiratory: Cough, nasal congestion, pharyngitis, sinus disorder, upper respiratory infection

Laboratory Test Findings

In controlled clinical trials, hyperkalaemia (serum potassium > 5,5 mEq/L) occurred in 1,5 % of patients, but in thes trials, discontinuation of COZAAR COMP due to hyperkalaemia was not necessary. Elevations of alanine amino trans ferase (ALT) have occurred but usually resolved upon discontinuation of therapy.

Hydrochlorothiazide

Other side effects that have occurred with hydrochlorothiazide are:

- *Gastrointestinal system:* anorexia, gastric irritation, nausea, vomiting, cramping, diarrhoea, constipation, jaun dice (intrahepatic cholestatic jaundice), pancreatitis, sialadenitis.
- *Central nervous system:* vertigo, paresthesias, headache, xanthopsia.
- *Haematologic:* leukopenia, agranulocytosis, thrombocytopaenia, aplastic anaemia, hemolytic anaemia.
- *Cardiovascular:* hypotension, including orthostatic hypotension (may be aggravated by alcohol, barbiturates, na cotics or antihypertensive drugs).
- *Hypersensitivity:* purpura, photosensitivity, rash, urticaria, necrotizing angiitis (vasculitis) (cutaneous vasculitis fever, respiratory distress including pneumonitis and pulmonary oedema, anaphylactic reactions.
- *Metabolic:* hyperglycaemia, glycosuria, hyperuricaemia, electrolyte imbalance.
- *Renal:* renal dysfunction, interstitial nephritis, renal failure.
- *Other:* muscle spasm, weakness, restlessness, transient blurred vision

Laboratory Test Findings

In controlled clinical trials with COZAAR COMP, hyperkalaemia (serum potassium > 5,5 mEq/L) occurred in 0,7 % patients, but in these trials, discontinuation of COZAAR COMP due to hyperkalaemia was not necessary. Elevations alanine amino transferase (ALT) have occurred but usually resolved upon discontinuation of therapy.

SPECIAL PRECAUTIONS

Hepatic and renal impairment

COZAAR COMP is not recommended for patients with hepatic impairment or severe renal impairment (see **DOSAG AND DIRECTIONS FOR USE**).

Hypotension and electrolyte/fluid imbalance

In patients who are intravascularly volume-depleted (e.g., those treated with high-dose diuretics), symptomatic hypoten sion may occur. These conditions should be corrected prior to administration of COZAAR COMP, or a lower starting dos should be used (see **DOSAGE AND DIRECTIONS FOR USE**). Periodic determination of serum electrolytes shoul be performed at appropriate intervals as in any patient receiving diuretics.
Other medication that affect the renin-angiotensin system may increase blood urea and serum creatinine in patients wit bilateral renal artery stenosis or stenosis of the artery to a solitary kidney. While not confirmed, this potentially may occu with angiotensin II receptor antagonists.

Metabolic and endocrine effects

Thiazide therapy may impair glucose tolerance. Dosage adjustment of antidiabetic agents, including insulin, may b required (see **INTERACTIONS**).
Thiazides may decrease urinary calcium excretion and may cause intermittent and slight elevation of serum calcium Marked hypercalcaemia may be evidence of hidden hyperparathyroidism. Thiazides should be discontinued before ca rying out tests for parathyroid function.
Increases in cholesterol and triglyceride levels may be associated with thiazide diuretic therapy. Thiazide therapy ma precipitate hyperuricaemia and/or gout in certain patients. Because losartan decreases uric acid, losartan in combina tion with hydrochlorothiazide attenuates the diuretic-induced hyperuricaemia.

Other

In patients receiving thiazides, sensitivity reactions may occur with or without a history of allergy or bronchial asthma Exacerbation or activation of systemic lupus erythematosus has been reported with the use of thiazides.

INTERACTIONS WITH OTHER MEDICAMENTS

Losartan Potassium

No medication interactions of clinical significance have been identified. Compounds which have been studied in clini cal pharmacokinetic trials include hydrochlorothiazide, digoxin, warfarin, cimetidine and phenobarbital.

Hydrochlorothiazide

When administered concurrently the following medication may interact with thiazide diuretics:
Alcohol, barbiturates, or narcotics: potentiation of orthostatic hypotension may occur.
Antidiabetic medication (oral agents and insulin): dosage adjustment of the antidiabetic drug may be required.
Other antihypertensive medication: additive effect or potentiation.
Cholestyramine and colestipol resins: absorption of hydrochlorothiazide is impaired in the presence of anioni exchange resins. Single doses of either cholestyramine or colestipol resins bind the hydrochlorothiazide and reduce it absorption from the gastrointestinal tract by up to 85 and 43 percent, respectively. COZAAR COMP should therefore b administered one hour before the intake of the resin.
Corticosteroids, ACTH: intensified electrolyte depletion, particularly hypokalaemia.
Pressor amines (e.g. norepinephrine): possible decreased response to pressor amines but not sufficient to preclud their use.
Skeletal muscle relaxants, nondepolarizing (e.g. tubocurarine): possible increased responsiveness to the muscl relaxant.
Lithium: should not generally be given with diuretics. Diuretic agents reduce the renal clearance of lithium and add high risk of lithium toxicity. Refer to the package insert for lithium preparations before use of such preparations wit COZAAR COMP.
Non-steroidal anti-inflammatory medication: In some patients, the administration of a non-steroidal anti-inflam matory agent can reduce the diuretic, natriuretic, and antihypertensive effects of loop, potassium-sparing and thiazid diuretics.

Effects on ability to drive and use machines

There is no data to suggest that COZAAR COMP affects the ability to drive and use machines.

KNOWN SYMPTOMS OF OVERDOSAGE AND PARTICULARS OF ITS TREATMENT

Losartan Potassium

Significantly lethality was observed in mice and rats after oral administration of 1000 mg/kg (3000 mg/m²) and 200 mg/kg (11800 mg/m²) (500 and 1000 times** the maximum recommended daily human dose), respectively.
Limited data are available in regard to overdosage in humans. The most likely manifestation of overdosage would b hypotension and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation. If symptomati hypotension should occur, supportive treatment should be instituted.
Neither losartan nor the active metabolite can be removed by haemodialysis.
***Based on a patient weight of 50 kg.*

Hydrochlorothiazide

The most common signs and symptoms observed are those caused by electrolyte depletion (hypokalaemia, hypochlo raemia, hyponatraemia) and dehydration resulting from excessive diuresis. If digitalis has also been administered hypokalaemia may accentuate cardiac arrhythmias. The degree to which hydrochlorothiazide is removed by haemodial ysis has not been established.

IDENTIFICATION

COZAAR COMP tablets are yellow, oval shaped, film-coated tablets, with '717' on one side and scored on the other side

PRESENTATION

COZAAR COMP tablets are supplied in blister packs of 30.

REGISTRATION NUMBER

30/7.1.3/0284

NAME AND BUSINESS ADDRESS OF THE APPLICANT

MSD (Pty) Ltd, 16th Road, Halfway House, 1685, Republic of South Africa.

Interview

A 'traditional' traditional healer: Philip Kubukeli

'It was the call of my ancestors,' says traditional healer Philip Sobanku Kubukeli, from Khayelitsha in the Western Cape, explaining how he came to this vocation. 'I had some symptoms which I could not understand, and my mother, who was a traditional healer, said they indicated that I was being called.'

And that call, which occurred when the Transkei-born and bred Kubukeli was in Standard 5, was to have a profound effect on his life, if for nothing else than curtailing his education. But now, more than 40 years on from that time, its power is no less strong as he devotes his energies largely to the quest for the recognition and respect for the profession that he believes it surely deserves.

Philip Kubukeli – on a quest for recognition for traditional healers.

Softly-spoken, Kubukeli explains that traditional healing is an inheritance and that in this tradition, he did his training as a healer under his mother, while at the same time completing his Junior Certificate. Subsequently he was sent to Lesotho for further training for 2 years with an 'elder' healer, under whom he qualified in 1962.

Such training essentially involves three components, he says. These are the spiritual training for diagnosis – 'you don't touch the patient but look at them and eventually the spirits of the ancestors will tell you from what the patient is suffering and the cause of it'; the dance, which 'encourages the spirits to come'; and a knowledge of the traditional medicinal plants, which occur throughout the country.

Kubukeli estimates that there may be up to 35 000 traditional healers in South Africa and possibly as many as 150 different healers' associations, and of these he says the Western Cape Traditional Healers and Herbalist Association, of which he is President, is both the largest – with some 1 500 members – as well as the most democratic, including all different types of healers.

But it is in such fragmentation that problems lie, he argues, and traditional healers are 'suffering'. They often feel left out in the cold by other medical practitioners and by government, and there is an increasing number of 'charlatans' who are practicing as healers. Further, these charlatans are putting traditional medicinal plants under threat by over-collecting them – in some cases, he says, in amounts of up to a hundred bags a week – and 'they are ultimately going to collect all the plants and there will be nothing left for future generations'.

The solution, Kubukeli believes, lies in the recognition by government of traditional healers and with this some form of control such as registration, and as a first step he has initiated discussions with a number of associations with a view to forming an umbrella body to 'help meet the objectives of all healers'. However, so far there has been only limited support from other associations, although he feels this may be due, in part at least, to a lack of understanding of the role of such a body.

He has also had some discussions with Western Cape MEC for Health and Social Services, Ebrahim Rasool, on the role of traditional healers and the formation of an umbrella body, but not as yet at national level. But, he says, 'I think Dr Zuma will be very understanding and I am trying to contact her.'

Nevertheless, Kubukeli feels that the gap between traditional healing and western medicine is narrowing and that efforts are being made by the two parties to work together. And this is something he tries to encourage, he says, pointing out that he himself is involved in a referral scheme where people with cancer or TB are referred on.

However, an issue that is of concern to traditional healers is the ownership of their knowledge and the fear that it may be 'stolen', he notes, adding that his view is that this should be 'input' to TRAMED (the traditional medicines research unit at UCT) and certain other bodies. Indeed he himself has supported the establishment of the unit, which will investigate the scientific basis of traditional healing, and says 'the chiefs of TRAMED have assured that our knowledge will be secure and won't reach other hands without our consent.'

Kubukeli believes that traditional healers have an important role to play in the health care system and that their numbers will grow in the future. And by way of example, he says that there are already some 200 white healers when formerly there were none.

In the meantime he is also getting involved in other projects to take traditional healing into the future, such as the drafting of a traditional medicines formulary with TRAMED and the cultivation of traditional medicinal plants in collaboration with organisations such as the Western Cape Nature Conservation department and the National Botanical Institute.

And, Kubukeli says: 'When we are recognised, we will then know we are working for the government and our people.'

Jonathan Spencer Jones

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Correspondence

Survival of human immunodeficiency virus-infected persons with pulmonary tuberculosis

We have described the radiological features of 150 HIV-1 infected patients presenting with pulmonary tuberculosis to a Cape Town HIV clinic between 1989 and 1994.[1] All patients had confirmed tuberculosis and received rifampicin-based short course chemotherapy (6 months RHZ for initial cases, 6 months RHZE for relapse cases). Treatment was both supervised (5 days per week) and self-administered (weekly supply of medication). Subsequent follow-up of this cohort demonstrated an overall mortality rate of 41/100 person years. However, different radiological presentations of pulmonary tuberculosis were associated with highly significant (logrank test $P < 0.001$) differences of Kaplan-Meier proportional survival (Figure). The total lymphocyte count was also found to be a major predictor of survival. Sixty-one percent of patients presented with lymphopaenia of <1250/μL, 39% had a total lymphocyte count >1250/μL. Lymphopaenic patients had a significantly higher mortality rate than those patients with a normal total lymphocyte count (65 vs 2/100 person years, χ^2 test $P < 0.001$).

The 2-year mortality rate of patients with HIV-1 infection and pulmonary tuberculosis described by Kassim et al.[2] was considerably lower (20.3/100 person years) than that reported by Small et al.[3] and ourselves. Each of these studies used rifampicin-based chemotherapy, but the different mortality rates could be explained on the basis of differences in study population. The CD4 lymphocyte count has been found to be a major determinant of mortality,[4] but is frequently unavailable in resource-poor countries. In contrast, chest radiographs and total lymphocyte counts are widely available and were powerful prognostic markers of mortality in our patients with HIV-1 related pulmonary tuberculosis. These variables have such a strong impact on mortality that they should be reported and controlled for in comparative studies of response to tuberculosis chemotherapy in HIV-infected individuals.

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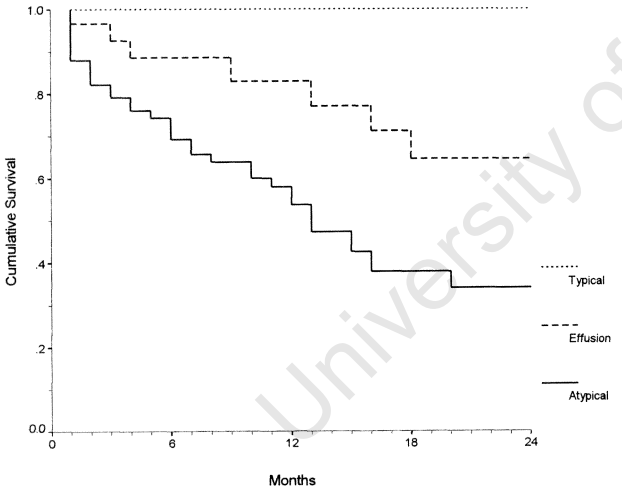

Number of patients at risk

Time (months)	0	6	12	18	24
Typical	18	15	10	6	5
Effusion	32	19	14	12	10
Atypical	100	52	27	14	10

Figure Kaplan-Meier estimated survival of HIV-infected patients with pulmonary tuberculosis, stratified by radiographic appearance. Typical presentation includes infiltrates limited to the upper lobes or apical segments of lower lobes. Effusion denotes pleural effusion. Atypical pattern includes lower and mid zone infiltrates, adenopathy and/or interstitial infiltrates. Survival is significantly impaired in those patients presenting with pleural effusion or atypical patterns (logrank test $P < 0.001$).

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Pulmonary clofazimine crystals in two patients with the acquired immunodeficiency syndrome

Mycobacterium avium complex (MAC) infections are frequent manifestations of the acquired immunodeficiency syndrome (AIDS).[1] They require combination therapy, often including a treatment used in leprosy, clofazimine. We report two cases of pulmonary crystals associated with clofazimine.

Case 1

A 31-year-old male patient with human immunodeficiency virus (HIV) infection known since 1988 received from November 1993 a combination of ethambutol 1 g q.d., clarithromycin 500 mg b.i.d. and clofazimine 100 mg b.i.d. for MAC bacteremia. Fever and hypoxemia improved until February 1994, when the patient was admitted for cough and dyspnea. Chest radiograph was unremarkable, and laboratory tests were similar to those observed the previous visit. Examination of bronchoalveolar lavage (BAL) revealed numerous red needle-shaped crystals in macrophages, evocative of clofazimine (Figure). Culture later yielded *Haemophilus influenzae.* Spirometric examination revealed a mixed ventilatory defect of moderate severity. Despite interruption of clofazimine, symptoms persisted. Spirometry performed 15 days later showed no improvement.

Case 2

A 34-year-old man with HIV infection known since 1987 received from March 1994 a combination of ethambutol 800 mg q.d., clarithromycin 500 mg b.i.d. and clofazimine 100 b.i.d. for MAC bacteremia. He was admitted in June 1994 for cough with purulent discharge and dyspnea. Chest radiograph showed an alveolar infiltrate of the left lower lobe. BAL examination revealed sparse macrophage crystals evocative of clofazimine. Culture yielded meticillin-resistant *Staphylococcus aureus.* Therapy was instituted with vancomycin and fusidic acid concomitantly with clofazimine. Pulmonary symptoms resolved in 7 days and did not recur in a 5-month follow-up.

In neither case was MAC isolated from blood or BAL culture at admission.

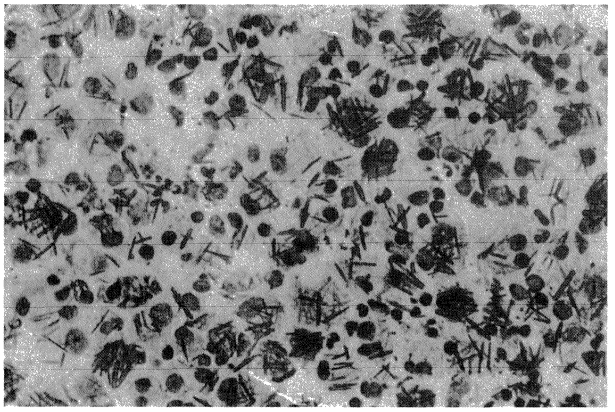

Figure Bronchoalveolar lavage specimen demonstrating numerous needle-like crystals (Giemsa, ×400).

Discussion

Clofazimine is a lipid soluble molecule which concentrates in the cells of the reticulo-endothelial system and macrophages.[2] High drug concentrations in the lungs and skin have been demonstrated.[2] Discoloration and crystal formation appear proportional to duration of therapy. Clofazimine is used in AIDS-associated MAC infection.[1] Three cases of clofazimine-associated pulmonary crystal in patients with AIDS have been published.[3–5] In two cases, clofazimine was pursued[4,5] without deleterious effects on pulmonary function. In the other[3] it was discontinued, although it is unclear whether symptoms were due to clofazimine or to MAC resistance to this drug. In our first patient, symptoms persisted despite interruption of clofazimine. In our second patient, pulmonary symptoms were clearly related to *S. aureus* pneumonia. The evolution, in our report, does not seem clofazimine-related. The appearance of crystals is probably only related to the duration of clofazimine therapy, and should therefore not prompt therapy interruption. The population of patients with MAC is expected to grow. The number of patients receiving clofazimine will probably increase even if clofazimine efficacy is decreased. These patients often present with pulmonary symptoms. Clinicians need to be aware of this complication and seek it with non-invasive methods such as induced sputum.[4]

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Levofloxacin in the treatment of drug-resistant tuberculosis

A 69-year-old male patient presented to the chest clinic under the administration of the Department of Health at the end of October 1994 with cough and haemoptysis. Chest radiograph revealed bilateral upper lobe cavitary lesions, and sputum microscopy showed presence of acid-fast bacilli. Thus active pulmonary tuberculosis was diagnosed. He had previously been treated for pulmonary tuberculosis in China in 1978 with streptomycin, isoniazid and rifampicin. The drug dosages used and compliance could not, however, be ascertained. In November 1994 the patient was started on a combination of kanamycin, isoniazid, rifampicin, ethambutol and pyrazinamide, given under full supervision. However the pretreatment sputum culture subsequently yielded *Mycobacterium tuberculosis* resistant to rifampicin, ethambutol streptomycin, kanamycin and amikacin, but susceptible to isoniazid, pyrazinamide, ofloxacin, ethionamide, and cycloserine. The patient was referred for treatment of his drug-resistant tuberculosis (DR-TB) in May 1995. He was started on isoniazid 300 mg, ofloxacin 600 mg, pyrazinamide 1.5 gm and also ethionamide 750 mg, all on daily basis. Cycloserine 750 mg once daily was added after one week of treatment. After receiving all drugs for four weeks the patient developed severe giddiness, followed by a generalized seizure. Serum ofloxacin was found to be 7.30 mg/L. Based on previous experience we felt that the patient's neurological reactions were due to the co-administration of ofloxacin and cycloserine.[1] Cycloserine was withdrawn, but the patient still complained of giddiness and unsteady gait. The peak serum ofloxacin level 2 weeks after the seizure episode, while the patient was still on ofloxacin 600 mg daily, was 9.74 mg/L. Ofloxacin was reduced to 500 mg daily in late June 1995, but the neurological symptoms did not improve. Because levofloxacin is more active in vitro against *M. tuberculosis* than ofloxacin (MIC_{90s} of $\leqslant 0.5 - 1$ mg/L versus 1–2 mg/L[2,3]), we substituted levofloxacin at a lower dose on the premise that this might lessen the risk of neurological reactions but preserve therapeutic efficacy. The patient was started on levofloxacin 300 mg, isoniazid 300 mg, pyrazinamide 1.5 g and ethionamide 750 mg, all once daily, at the beginning of September 1995. One week after switching from ofloxacin to levofloxacin his condition improved, with giddiness and unsteady gait subsiding almost completely. Subsequently, isoniazid was also withdrawn because the sputum culture of June 1995 also showed resistance to isoniazid. In October 1995 he was discharged, free from any neurological symptoms, on directly observed therapy with levofloxacin, ethionamide and pyrazinamide. Sputum cultures collected in July, August, September and October 1995, as well as those from March, May and July 1996, were all negative. The chest radiographs were also stable. As closure of cavities had not occurred by July 1996, the treatment was continued for a total of 18 months.

This case suggests the potential utility of levofloxacin in the face of adverse neurological reactions to ofloxacin. Levofloxacin, more active than ofloxacin against *M. tuberculosis* both in vitro and in vivo,[2–4] might be used for treatment of DR-TB at lower doses than ofloxacin. This could reduce the risk of neurotoxicity and other adverse reactions. However, more clinical data need to be accrued before levofloxacin can firmly be recommended as a preferred drug over ofloxacin.

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Short-course chemoprophylaxis evaluated in gold miners with chronic silicosis

In the paper entitled 'Short-course chemoprophylaxis with rifampicin, isoniazid and pyrazinamide for tuberculosis evaluated in gold miners with chronic silicosis: a double-blind placebo controlled trial' by R L Cowie (Tubercle Lung Dis 1996; 77: 239–243), the author concludes that the regimen failed to prevent tuberculosis. Since the medication was self-administered,

CORRESPONDENCE

Cutaneous Miliary Tuberculosis in the AIDS Era

SIR—A recent review of cutaneous miliary tuberculosis in the AIDS era [1] emphasized the importance of having a high index of suspicion for this condition in HIV-positive patients with skin lesions and advanced immunodeficiency. However, the authors were able to identify only five such cases in the literature, in addition to the case they reported. In a prospective study, ongoing since October 1994, we have identified only one case of cutaneous tuberculosis among nearly 400 patients with biopsy-confirmed or culture-confirmed tuberculosis; 47% of these patients were HIV-positive. In view of the rarity of cutaneous tuberculosis and the fact that skin biopsy is not usually necessary to make a diagnosis of disseminated tuberculosis, we believe that skin biopsy should be performed primarily to exclude other causes of skin conditions in patients with advanced HIV disease, as the following case report illustrates.

A 48-year-old HIV-infected heterosexual man who had no history of use of illegal substances but who had received treatment for two previous episodes of tuberculosis presented with chronic pathogen-negative diarrhea, colicky abdominal pain, weight loss, fever, and dark maculopapular lesions on his hands. The CD4 cell count was 3×10^6/L, and a chest roentgenogram showed fibrocystic changes in the right upper lobe and possible mediastinal adenopathy.

An ultrasonogram of the abdomen showed adenopathy in the epigastrium. No sputum was obtained, but a blood culture for mycobacteria with use of the radiometric system (BACTEC; Becton Dickinson, Sparks, MD) was negative. Examination of a duodenal biopsy specimen obtained by endoscopy revealed cryptosporidia. One of the skin lesions was biopsied to exclude Kaposi's sarcoma, and the specimen was sent for a routine mycobacterial culture. A diagnosis of disseminated tuberculosis was made on the basis of the patient's clinical presentation and the presence of adenopathy; he was discharged and received antituberculous therapy as an outpatient. *Mycobacterium tuberculosis* was cultured from the skin biopsy specimen 2 weeks later.

While cutaneous manifestations are common in the HIV-positive population in Cape Town, South Africa, few lesions are biopsied, so we may have missed other cases of cutaneous miliary tuberculosis. However, as was true for our patient, the diagnosis of disseminated tuberculosis is usually evident on clinical grounds; cultures are performed to confirm the diagnosis and to exclude drug resistance. Although the positive culture of the skin biopsy specimen confirmed the diagnosis for our patient, the result was unexpected; if the result had been negative, treatment would have been continued. Similarly, in the case reported by Libraty and Byrd, the patient's risk factors and presentation, apart from the short history of symptoms, were suggestive of disseminated tuberculosis. Further, the positive culture result for the skin biopsy specimen became available after acid-fast bacilli had been seen on examination of bronchoalveolar lavage fluid and bone marrow biopsy specimens.

In conclusion, our own experience and that of other investigators [2] is that an HIV-related diagnosis of extrapulmonary tuberculosis can usually be made without performing a skin biopsy. Notwithstanding the findings in two recent reports [3, 4], in most cases it would be more appropriate to use procedures with documented high diagnostic yield, e.g., lymph node biopsy [5], than to perform a skin biopsy. Isolated instances of positive skin biopsy specimens must be weighed against the large number of negative skin biopsy specimens if the recommendations of Libraty and Byrd are followed.

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Clinical Infectious Diseases 1997;25:1484

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Cutaneous Miliary Tuberculosis in a Patient Infected with Human Immunodeficiency Virus

SIR—Libraty and Byrd [1] recently described a case of cutaneous miliary tuberculosis in an HIV-positive patient and reviewed the published cases of cutaneous miliary tuberculosis among patients with AIDS [1]. The authors claimed that only six cases (including their own case) have been reported so far. However, they did not mention two cases of cutaneous miliary tuberculosis due to multidrug-resistant *Mycobacterium tuberculosis* in patients with AIDS, which we described previously [2]. Since the publication of our report in October 1995, we have diagnosed a further case of cutaneous miliary tuberculosis, also known as tuberculosis cutis miliaris disseminata (TCMD).

A 38-year-old man with an 8-year history of intravenous drug abuse who was receiving methadone maintenance therapy presented with a 1-week history of high fever (temperature, 40°C), chills, a nonproductive cough, epigastric pain, and dysphagia. He had been aware of his HIV seropositivity for 3 months. He was not receiving any antiretroviral therapy or prophylaxis for *Pneumocystis carinii* pneumonia.

SAMJ
Letters to the Editor

Briewe

The SAMJ and editorial independence

To the Editor: It was with a sense of pleasure and vindication that I read the June issue of the *SAMJ* dealing with the MASA's submission to the TRC, and I congratulate the Editor on at last publishing the letters received by the *SAMJ* at the time of the Biko affair. I leave it to others better qualified than I am to decide how best the MASA can continue to shake off the legacy of its dubious past and plan for the future as a major guardian of the conscience of the profession. However, I feel that I must at this juncture put forward a strong plea for editorial independence of the *SAMJ* from its MASA proprietors, a process already started, but one which needs to be clearly recognised as a crucial issue if the mistakes of the past are never to be repeated.

When I joined the *SAMJ* in 1983 as Assistant Editor, the Editor was firmly under the control of the MASA hierarchy, and was not allowed to print anything remotely controversial without reference to either the Secretary-General or the Chairman of Federal Council. At the time I considered this to be a wholly unsatisfactory state of affairs, and one which could only lead to the *SAMJ*'s never being able to function as the voice of the whole South African medical profession, and not just that of the MASA's current masters.

When I became Editor in 1987, I tried to move the *SAMJ* in the direction of editorial independence — a process akin to walking on eggs, but which, I believe, worked, or at least started to. In this I was greatly inspired by the example of the *BMJ*, which made its bid for editorial independence from the BMA when Hugh Clegg was Editor. At a time when the BMA was rather sycophantically wooing the Royal Colleges, Clegg wrote an editorial entitled 'The gold-headed cane' which fired a torpedo into the whole process. At the subsequent meeting of the BMA Council, a motion was put to fire him, but, to its everlasting credit, the Council gave Clegg its support in the interests of free speech. The *New England Journal of Medicine* also has a proud record of editorial independence, and Arnold Relman, the previous editor, wrote that never, throughout its whole existence, had the Massachusetts Medical Society attempted to interfere with editorial decisions. This is probably one of the major reasons for its great reputation.

Both the MASA and the *SAMJ* have come a long way in the past 10 years, but the issue of editorial independence needs to be recognised as a vital ingredient in their mutual relationship. Like the stripes of a zebra, both belong together, but to confuse their functions can only lead to a monotonous and ineffectual shade of gray. As Stephen Lock once wrote in the *BMJ*, nobody wants to publish in a journal which is a constrained parish magazine.

Nick Lee
Emeritus Editor, SAMJ

Promoting HIV vaccine research and development in southern Africa

To the Editor: In a recent editorial[1] we proposed that a working group be established to promote HIV vaccine research in Africa. In follow-up, we wish to report on a workshop held in Cape Town on 13 March 1997 as part of the 3rd IUIS African Immunology Conference concerning promotion of HIV vaccine development and research in Africa. The workshop brought together more than 50 AIDS researchers from 9 African countries (Tanzania, South Africa, Zimbabwe, Nigeria, Kenya, Uganda, Mozambique, Ethiopia and Cameroon) as well as from Europe and the USA to present data and discuss ideas on what else is needed to accelerate the development and evaluation of HIV vaccine(s) for use on the African continent. Representatives from three international organisations with the mandate for promotion of HIV vaccine research, the HIV Network for Prevention Trials (HIVNET, a USA National Institutes of Health Program), the International AIDS Vaccine Initiative (IAVI) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) also participated. While the workshop focused predominantly on basic science, other issues relating to ethics, conduct of field trials and advocacy and lobbying for vaccine development were touched upon to highlight some of the gaps in knowledge that currently exist. Of a total of more than 24 million people living with HIV/AIDS in the world, 18.5 million are sub-Saharan Africans[2] and more than 2 million are South Africans (R Swanevelder, Department of Health, South Africa — personal communication). The continued spread of HIV makes development of vaccines an increasingly important part of current prevention strategies.

Most candidate HIV-1 vaccines to date are based on the genetic subtype B of HIV-1. However, the HIV-1 subtypes prevalent in Africa are mostly from subtypes other than B, namely A, D and E in west, east and central Africa and C in southern Africa. At present the immunological significance of this genetic variation is not clear. Preliminary data presented at the workshop suggest limited cross-reactivity of neutralising antibodies directed to different HIV-1 subtypes. On the other hand, cell-mediated immune responses against different HIV antigens seem to be more cross-reactive. Overall, this emphasises the need to obtain additional information on the possible protective efficacy of experimental vaccines in populations infected with different HIV-1 subtypes. Further laboratory-based research and efficacy trials are needed to obtain such information. In preparation for future HIV vaccine trials in Africa, it is important to build and strengthen capacity for laboratory, clinical, epidemiological and social-behavioural vaccine-related research, including the establishment and follow-up of well-characterised cohorts of HIV-1 seronegative

volunteers. (Epidemiological research capacity building has already been initiated in South Africa through the Fogarty International HIV/AIDS Research and Training Program. Since its inception in 1993, eight South African epidemiologists have been trained at master's level at Columbia University, New York (Grant Two-0231).)

Vaccine research in humans should adhere to the strictest possible code of ethics to ensure that volunteers are willing to participate freely in trials, that they will not be subjected to unnecessary risks (medical or social), and that individuals and populations will benefit from the results of the research. It is clear that the issues are many and complex. The need for rapid exchange of information and for a working group to guide informed decision-making is obvious.

The authors are keen to initiate such a group in South Africa, with membership open to anyone interested. To initiate the process of facilitating channels of communication between AIDS researchers in southern Africa, and their colleagues elsewhere, it was proposed that a web site and electronic mailing list be created for the analysis and subsequent dissemination of information. This will address vaccine development and other HIV research issues relevant to Africa, and will be effected by the South African National Bio-informatics Institute at the University of the Western Cape. Any person wishing to participate or provide information can contact the following people: Elna van der Ryst (gnvrevdr@med.uovs.ac.za), Win Hide (winhide@techno.sanbi.ac.za), Quarraisha Abdool Karim (sakarim@hoopoo.mrc.ac.za) or Carolyn Williamson (cwilliam@medmicro.uct.ac.za). This activity will be co-ordinated with the HIV vaccine trials network being established by UNAIDS and with the IAVI.

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Diagnostic disagreement — the lessons learnt from malaria diagnosis in Mpumalanga

To the Editor: Hansford and Van Vuuren[1] should be commended for implementing a training and quality assurance programme for microscopists, as recommended in our article.[2] However if, as they assert, repeated examination with loss of material and fading was the principal explanatory variable for our finding of malaria diagnostic disagreement, the positivity rate would be expected to decline at consecutive laboratories. However, no such trend was found. Consecutive examination by the first, second, third and fourth examining laboratories recorded 8.3%, 45.8%, 6.3% and 20.8% positive blood smears, respectively ($\chi^2_{trend} = 0.0$, df = 1, $P > 0.5$).

The focus on laboratory 2 is unfounded, as our statistical analysis demonstrated very poor concordance between all of the six possible laboratory pairs.[3]

Although the polymerase chain reaction promises to provide a gold standard for malaria diagnosis in the future, its ability to detect sub-microscopic parasite burdens could complicate inter-laboratory comparison further.

The object of the present investigation was to explore the quality of microscopy of routine field blood smears. We agree that blood smear preparation by busy clinics is often inadequate, thus complicating microscopy. We therefore recommend that where cost-effective valid alternatives for rapid malaria diagnosis are available, these should replace routine microscopy for field diagnosis.

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1. Hansford CF, Van Vuuren CM. Diagnostic disagreement with malaria diagnosis in Mpumalanga (Letter). *S Afr Med J* 1997; **87:** 910-912.
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Conscious sedation in children

To the Editor: The publication of the conscious sedation clinical guidelines[1] by the MASA Conscious Sedation Working Group is commendable. However, it has failed in its objective of global application in that the focus is narrow, particularly with regard to conscious sedation in children. It is unfortunate that neither the Association of Paediatrics nor the Association of Paediatric Surgeons was invited to contribute.

Conscious sedation in children may be indicated for a variety of procedures other than those mentioned in the

guidelines, i.e. diagnostic imaging (CT, MRI) or endoscopy. These include lumbar punctures, bone marrow biopsies, suturing of lacerations, reducing fractures, dressing changes (particularly for burns), chest tube placement or removal, echocardiography or cardiac catheterisation, to mention but a few, and involve every discipline. Indeed even blood sampling can be extremely traumatic for a child and may require sedation. Each of the above procedures will require a varying degree of sedation depending on the age, health and character of the child, the parents' views and the attitude and character of the 'sedationist' (*sic*).

The glib remark that children require a 'deeper degree of sedation' and that 'before embarking on repeat doses' or additional sedatives an 'anaesthesiologist should be consulted' is dangerous. Deeply sedated children can easily move to a state of general anaesthesia depending on individual response, route of drug administration and degree of stimulation. Paediatrics-trained individuals capable of monitoring and resuscitating a neonate, infant, toddler or child should be in attendance both during and after the procedure. Appropriate equipment for that particular child's age group should be available prior to initiation of the sedation, particularly in institutions where children are not often treated. Conscious sedation in children should not be undertaken lightly. Despite adherence to the guidelines for paediatric patients by various interest groups,[2-5] which the authors recommend as essential reading, conscious sedation carries significant morbidity and mortality,[6,7] particularly when opiates are used.[8]

A sedation treatment plan should be formulated to handle any eventuality prior to, during and after the procedure — with an anaesthesiologist or someone equally capable on standby if the situation so dictates. Psychological support techniques (cuddling, parental support when appropriate, warm blankets, a gentle reassuring voice, hypnosis) are useful adjuncts and often forgotten outside the paediatric environment.

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1. MASA Conscious Sedation Working Group. Conscious sedation clinical guidelines. *S Afr Med J* 1997; **87:** 484-492.
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Professor D Morrell, Dr E Barker and Ms V J Pinkney-Atkinson reply: We thank Professors Bosenberg and Hadley for their contribution and comments. First, it is common knowledge that conscious sedation in children is a far more risky undertaking than in adults and should not be undertaken by anyone inexperienced in paediatric pharmacology. We agree that children require special management during conscious sedation and the lack of this information in the guideline is apparent. Participants at conscious sedation workshops repeatedly request more information on paediatric patients.

Second, the conscious sedation clinical guideline development process made adequate provision for wide consultation. The South African Association of Paediatrics and the South African Association of Paediatric Surgeons were invited to comment in a letter sent to them on 22 November 1996. Neither organisation returned the endorsement/comment form. Only the Radiological Society of South Africa made reference to children in its comments. Individuals were invited to comment on the guideline via the MASA *Medigram* publication of 9 November 1996. Some 50 doctors responded and asked for the draft guideline.

The following steps have been taken:

1. The existing conscious sedation clinical guideline will be publicised and labelled as pertaining to 'adults only'.
2. A separate evidence-based paediatric conscious sedation clinical guideline will be developed before the end of 1997. It is hoped that Professors Bosenberg and Hadley will be key participants in this process, as will other experienced practitioners. Discussions to this effect are already under way. Further details will be published in *Medigram*.

Reconciliation and justice

To the Editor: I returned from a 22-year exile to help build the new South Africa and have faced considerable disappointment in the last 3 years. Being excluded from posts I applied for because of being an elderly white man without ANC endorsement was acceptable as evidence of transformation. Working in a health service which continues to ignore the needs of the rural and dispossessed has been a very sore point, but the health service will continue to be pressurised to get its priorities right.

My biggest concern has been the lack of interest in this process of reconciliation by the medical profession and the previously privileged public. The handful of activists of the past remain a concerned handful while the voices of the previously compromised, who now apologise for the past, sound very hollow and insincere. No sacrifices are being made to atone for being a beneficiary of apartheid, and no one that I know of has volunteered to do community service in deprived areas. Even our Health Minister is too timid to demand such conscription from the pre-1990 graduates and prefers to tackle inexperienced and innocent young doctors.

I did not seek reconciliation. My own resistance to apartheid (of course, these days we *all* fought apartheid) was on a small scale which reaped massive punishments. My career was damaged by dismissals from two hospitals as a houseman. When I asked the MASA for help, they suggested I emigrate to the USA. Only one of my professors, Ossie Heyns, gave me any sort of support. I worked as a mine medical officer until I was arrested in July 1964 and my pregnant wife was evicted a week later. I was

in jail when my beloved father died and I was not allowed to attend his funeral. My son was born when I was in jail and I was never allowed to touch him until my release when he was nearly 2 years old. I was banned and even the University of the Witwatersrand would not give me a job at Alexandra Clinic in 1966. The Minister of Justice (*sic*!) denied me the right to study for my Diploma in Public Health at Wits — and even just to go to the library to read journals! I finally left for Edinburgh on an exit permit in 1968, painfully leaving my stepson behind to go to boarding school.

Who can make up for these things? Hundreds of thousands of such things occurred to tens of thousands of people. Millions experienced daily humiliations. Will we ever hear about them? The answer has to be 'no'. And the expressions of regret from the innocent beneficiaries of apartheid are very faint, even in the humane medical profession.

Real transformation remains on the horizon. Doctors can contribute to the process by regarding themselves as Africans and giving their loyalty to Africa, rather than to their pockets. This is the spirit that will create the much-vaunted 'rainbow nation' that does not yet exist.

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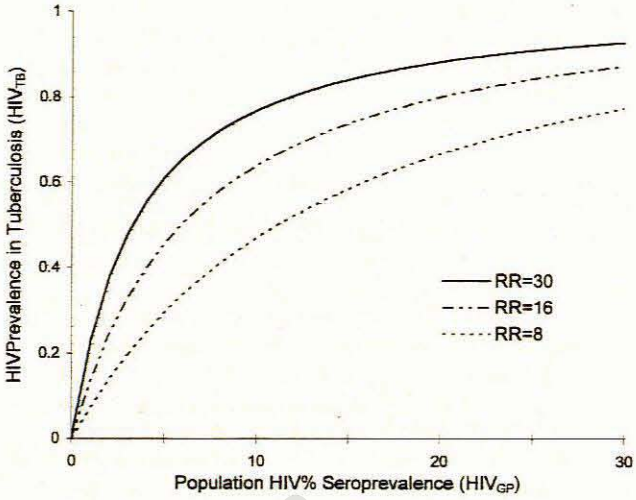
HIV-related tuberculosis

To the Editor: Wilkinson and Davies recently described the changing epidemiology and clinical features of tuberculosis in a rural KwaZulu-Natal hospital and related this to a rapid increase in HIV seroprevalence in antenatal clinics in the region.[1] We have also noted an increase in tuberculosis cases at Somerset Hospital, Cape Town, where 21% of admissions of black patients to the general medical wards are now for tuberculosis. Atypical clinical and radiological presentations have also become more common.[2,3]

Some comment is needed on the authors' estimate of the proportion of tuberculosis cases attributable to HIV infection. The authors estimated the attributable fraction (AF) of tuberculosis due to HIV infection using the relative risk (RR) of HIV infection in patients with tuberculosis compared with antenatal women. Ideally, the RR used in the calculation of AF should have been the ratio of incidence of tuberculosis in a cohort with or without HIV infection. Given the difficulty in obtaining such data, the RR for tuberculosis in HIV infection can be derived from the relationship beween HIV_{TB}, the proportion of HIV infection in tuberculosis, and HIV_{GP}, the general population's HIV seroprevalence. Using this relationship (Fig. 1) and assuming that HIV prevalence in antenatal clinics (HIV_{AN}) is equivalent to HIV_{GP}, the calculated RR for tuberculosis in the HIV-positive population in KwaZulu-Natal is 8.5, somewhat higher than the quoted 4.14.

Although it is generally assumed that the RR for tuberculosis in HIV-positive people is constant in different settings, this is unlikely, firstly because of the variation of risk of reactivation with immune status of the individual and



In a defined population, the proportion of tuberculosis cases that are HIV-positive (HIV_{TB}) is given by the ratio of cases of HIV-positive tuberculosis to the total number of cases. (Where HIV_{TB} is the proportion of HIV infection in tuberculosis cases, TB is the tuberculosis incidence rate and HIV_{GP} is the HIV seroprevalence in the general population.

$$HIV_{TB} = \frac{(HIV_{GP} \times TB \times RR)}{(HIV_{GP} \times TB \times RR) + (TB \times (1-HIV_{GP}).}$$

HIV_{TB} is therefore independent of tuberculosis incidence rate (TB) and is a function of HIV_{GP} and RR.

$$HIV_{TB} = \frac{(HIV_{GP} \times RR)}{1+HIV_{GP}\ (RR-1).)}$$

Fig. 1. Plots of HIV prevalence in tuberculosis versus general population HIV seroprevalence are shown for three groups at differing RRs for the development of active tuberculosis — Hlabisa patients with an RR of 8, Cape Town patients with an RR of 16 and AIDS patients exposed during a tuberculosis outbreak with an RR of 30.

hence the stage of the epidemic in the population and, secondly, because of the varying risk of transmission of tuberculosis from and to HIV-infected adults, depending on behavioural and socio-economic factors. At Somerset Hospital, a new diagnosis of HIV infection was made in 45% (CI 32 - 58%) of black women admitted with tuberculosis in 1995 - 1996. During this period, the antenatal HIV prevalence for this population group was 4.7% (CI 2.5 - 6.9%). Fig. 1 shows a plot of HIV_{TB} versus HIV_{GP} for KwaZulu-Natal with an RR of 8, for Cape Town where the RR obtained using the formula is 16 and for AIDS patients with an RR of 30, calculated during a tuberculosis outbreak.[4] The Cape Town analysis was limited to black females between the ages of 14 and 49 years with a new diagnosis of tuberculosis, for whom the HIV_{AN} should best approximate the HIV_{GP}. Patients with prior known HIV-positive status were excluded from the analysis to avoid referral bias from local HIV clinics. It should be noted that all curves show an initial steep slope which moderates with increasing seroprevalence. This non-linear relationship illustrates why tuberculosis patients act as a sentinel group for HIV early in the epidemic, all the more so if the RR for tuberculosis in HIV-positive patients is high. The initial steepness of the curves, however, should caution us that inaccuracies in HIV_{GP} will be reflected as large changes in RR calculated with the formula, especially when HIV_{GP} is < 20%. Calculation of attributable risk further amplifies any inaccuracies in the measured RR inherent in this type of analysis.

Both the tuberculosis incidence rate and the RR associated with HIV infection in KwaZulu-Natal are similar to other reports from Africa.[5] In contrast the high RR of the Cape Town urban black population may indicate a significant amount of horizontal transmission. Combined with a background tuberculosis incidence rate that is already the highest in Africa, we can expect an increase in HIV-related tuberculosis in Cape Town that is unprecedented in the global HIV epidemic to date.

Finally, we would like to emphasise the point made by Wilkinson and Davies, that national notifications have not increased in recent years.[6] The lack of culture facilities in most areas of South Africa, the high rate of smear-negative cases and the atypical presentation in advanced HIV infection are probable causes of underreporting. However, it must also be questioned whether the widespread underreporting is a manifestation of the crisis of morale and manpower in our health care system. Studies such as that of Wilkinson and Davies complement routine notification, but should not be a substitute for it. Population attributable fraction and RR may be considered abstruse, but accurate measurement of these parameters is important for projection of the impact of the HIV/tuberculosis epidemic in South Africa.

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To the Editor: I read with great interest the paper by Wilkinson and Davies[1] describing the increasing burden of HIV-related tuberculosis. The importance of their message is beyond any doubt. I would like to add two points. Firstly, the authors seem to be unaware of my previous report,[2] which, as far as I am aware, is the first study on the impact of the HIV epidemic on tuberculosis in rural South Africa. Of much more importance, however, is the fact that both surveys[1,2] point in the same direction.

Directly observed therapy (DOT) was started at Emmaus Hospital, Bergville District, KwaZulu-Natal, in 1987.[3] A survey from 1987 through 31 July 1995, showed a 3.7-fold caseload increase, i.e. in excess of Hlabisa's. Smear positivity rate was much higher — 91% without known or suspected HIV and 64% with HIV. The HIV status was tested in consenting patients in whom, on a clinical basis, there was a suspicion of their being immunodepressed. They represented 11.2% of all tuberculosis patients enrolled for DOT between 1 August 1994 and 31 July 1995. The proportion of HIV seropositivity was 71.4%, i.e. in excess of 55% at Hlabisa in 1995. The overall mortality rate increased over the years from 3% in 1987 to 11% during the last year of the survey. The overall tuberculosis reactivation rate was 13.7%; the estimated prevalence of drug resistance was 6%. (No figures concerning same are reported from Hlabisa.) The RR of HIV infection with tuberculosis was 5.1; the attributable fraction where tuberculosis was attributable to HIV was 0.80. At Hlabisa the latter two values were 4.14 and 0.76, respectively.

In conclusion, the similarities between both reports are striking, and confirm the magnitude of the problem of HIV-related tuberculosis in rural South Africa. I also agree with the authors that DOT is the most cost-effective management of tuberculosis.

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Dr Wilkinson replies: We are grateful for the interest in our work shown by your correspondents. We did not quote Van Bogaert's work as the results are probably biased and should be interpreted cautiously. If HIV testing is limited to patients in whom immunosuppression is suspected, reported HIV seroprevalence will most probably be high. Indeed only 11.2% of patients seem to have been tested: it is therefore not possible to draw conclusions about the HIV status of other patients, and further analysis should be undertaken cautiously. We have previously reported the data requested.[1]

The formulas used by Wood and Hudson are not referenced and we are not sure that all their assumptions hold. However, we did state in our paper that our estimate of the population attributable risk percentage was probably conservative and we gave reasons and justification for this. We need to be cautious about drawing broad assumptions from referral settings, as it is very difficult to be sure what the source population really is and how unbiased patient selection is. It is not clear what criteria are used for admitting patients with suspected tuberculosis to Somerset Hospital. It is also not clear if there are substantial differences in tuberculosis transmission rates between Cape Town and KwaZulu-Natal. In a large community-based study of the molecular epidemiology of tuberculosis transmission[2] we showed that approximately 45% of new cases of smear-positive tuberculosis were probably recently transmitted, which is a higher proportion than that shown by Warren *et al*. in Cape Town.[3] We await with interest unbiased, population-based data from Cape Town that document accurately the extent of the impact of HIV infection on tuberculosis.

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Occurrence of human herpes virus 8 in Kaposi's sarcoma and other tumours in South Africa

To the Editor: Kaposi's sarcoma (KS) is becoming the commonest tumour in populations with high seroprevalence rates of HIV.[1] In Uganda 48% of cancers in males and 20% of those in females are now KS, compared with less than 2% 20 years ago.[1] Increases in the incidence of KS attributed to the HIV epidemic have also been noted in Zimbabwe[2] and in Zambia.[3] No increases in the incidence of KS have been reported by the South African National Cancer Registry (NCR) up to 1991, but anecdotal unpublished data from the NCR suggest that KS is now seen more frequently. In a recent study in South Africa, the relative risk (odds) of an HIV infected person's developing KS was about 60.[4]

The non-random distribution of KS in HIV-infected individuals in the West (i.e. common in homosexual men, but uncommon in patients with haemophilia or intravenous drug users) has suggested that a sexually transmitted agent could be involved in the pathogenesis.[5] Recently, sequences of a new gammaherpesvirus, designated Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8), were identified in AIDS-KS biopsies, and have since been found in all epidemiological forms of KS (classic, endemic and post-transplant).[6-8]

We used the polymerase chain reaction (PCR) to test for the presence of HHV8 DNA in South African paraffin wax-embedded tissues from 10 KS (5 HIV-positive and 5 HIV-negative) and 70 non-KS tumour specimens. Specimens were retrieved from the South African Institute of Medical Research (Department of Anatomical Pathology) archives. DNA was extracted from 10 µm tissue for PCR for HHV8 as previously described.[9] PCR products (233 bp) were electrophoresed and visualised on a 3% agarose gel stained with ethidium bromide.

HHV8 was initially detected in 8 of 10 KS biopsy specimens (80%) and in 4 of 70 non-KS tissues (5.7%) (Table I). We tested the quality of DNA in the 2 HHV8-negative KS specimens using PCR to amplify human pyruvate dehydrogenase (HPD). This was negative, indicating that this DNA was not adequate for PCR. All of 8 KS biopsy specimens were therefore positive for HHV8. Assuming that the 70 non-KS tumours are an adequate unmatched control group, the relative odds of the association between HHV8 and KS was 251.2 (95% confidence interval 12.4 - 5 084.9) ($\chi^2 = 49.0$, $P = 0.001$).

In this study of South African samples we confirm the findings of others that HHV8 DNA is present in HIV-positive and negative cases of KS, but seldom detectable in other tumours or tissues.[6-13] This indicates that either HHV8 is specifically associated with KS or HHV8 is a passenger virus ('opportunist') in KS tissue because the virus, or the cell type harbouring HHV8, preferentially replicates in the cytokine-rich micro-environment of KS. First-generation serological assays, however, suggest that HHV8 is not ubiquitous, but predominantly present in those at risk for developing KS.[14-16] These early serological studies therefore support a causative role for the new virus in KS pathogenesis.

Table I. Prevalence of HHV8 in tissue specimens of KS and other tumours

Tissue	HHV8-positive/total
KS	
HIV positive	5/5
HIV negative	3/5 (/3)*
Total KS	8/8 (100%)
Non-Hodgkin's lymphoma	1/10
Nasopharyngeal tumours	1/10
Ovarian tumours	1/10
Colorectal tumours	1/10
Breast tumours	0/10
Hodgkin's lymphoma	0/10
Non-malignant tumours	0/10
Total non-KS	4/70 (5.7%)

* DNA was amplifiable in HHV8-negative specimens (see text).

Serological surveys based on an immunofluorescence assay (IFA) detecting an HHV8 latent antigen have shown that the prevalence of HHV8 varied from about 1.4% in British and American blood donors to 80 - 100% in AIDS-KS and classic KS patients.[14-16] In Ugandan HIV-negative individuals, depending on the assay, HHV8 seroprevalence rates ranged from 35% to 53%. In the pre-AIDS era, KS showed a marked difference in geographical distribution in Africa, being commoner in the tropics than in the north or south,[17] and if HHV8 is the cause of this tumour one would expect the seroprevalence rates in the different countries to reflect this. It would be important to measure the seroprevalence of HHV8 in South African populations of varying HIV risks to inform projections about the increasing incidence of KS in the country and to look at risk factors facilitating its transmission. We are currently conducting transmission and serological studies in South Africa to address some of these questions.

This study was approved by the University of the Witwatersrand Ethics Committee and funded by the Freddy Mercury Trust Fund and the Institute of Cancer Research, London. The National Cancer Registry is funded by the Department of Health, the Cancer Association of South Africa and the South African Institute for Medical Research.

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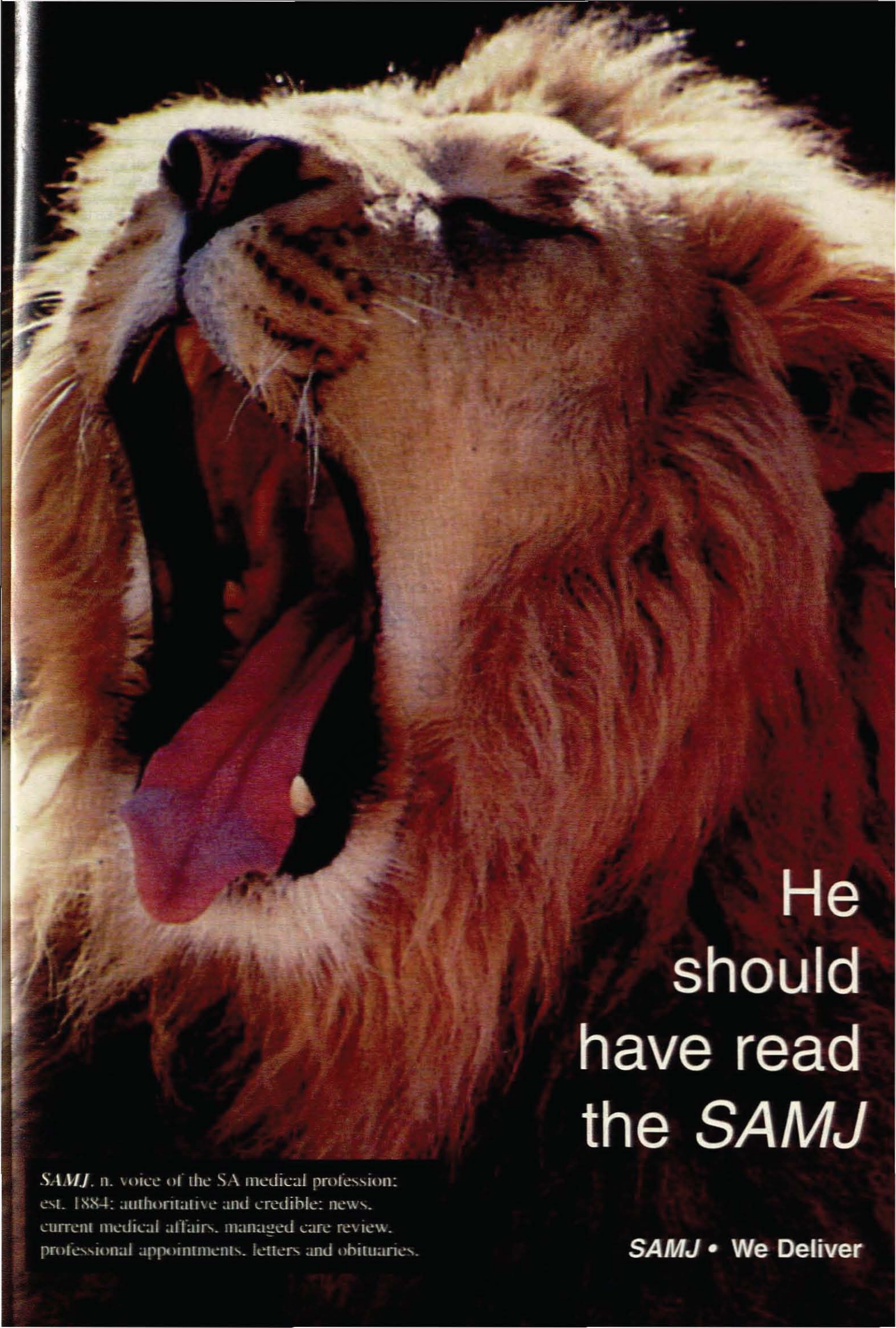

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Evaluating community radio as a medium for health messages in Alexandra

To the Editor: Like any evaluations of media-based health messages that compare 'exposed' (readers, listeners or viewers) and 'unexposed' (non-readers, non-listeners or non-viewers) individuals, Bardwell *et al*.'s[1] evaluation of Radio Zibonele as a medium for health messages relied on three inherent assumptions: (*i*) that exposure to additional sources of health information was the same for 'exposed' and 'unexposed' groups; (*ii*) that these groups were comparable in terms of educational and socio-economic characteristics that influence access to health information; and (*iii*) that the initial health knowledge of each group was the same. The limited validity of all three assumptions became apparent during an evaluation of Soul City's 'Healing Hearts' programme which was broadcast on Alexandra's community radio station, Alex FM, from October 1995 to January 1996. This 60-part series, which included a weekly phone-in discussion programme, covered a variety of topics including pregnancy, child care, breast-feeding and immunisation. Interviews with 250 women resident in Alexandra (81 listeners and 169 non-listeners), conducted 8 weeks after the broadcasts ended, revealed that significantly more of those who listened to 'Healing Hearts' had previously watched television broadcasts of Soul City (61.7% of listeners v. 43.2% of non-listeners; $\chi^2 = 7.52$, df = 1, $P = 0.006$), and that listeners were significantly more likely to have seen the booklet that accompanied Soul City (63.0%) than non-listeners (40.8%; $\chi^2 = 10.75$, df = 1, $P = 0.001$). In view of the greater impact of health campaigns that use multiple exposures in different media ('multimedia'),[2] it was surprising that these listeners did not select a higher percentage of correct answers in a 20-item questionnaire based on topics covered by the 'Healing Hearts' series (listeners: SD 76.7 ± 17.5%; non-listeners: SD 77.2 ± 16.0%). However, because the programme was targeted at low-income women, aged 15 - 45 years,[2] it is possible that there were pre-existing differences in health knowledge between listeners and non-listeners. If, for example, the listeners had poorer initial health knowledge than non-listeners, then the comparable questionnaire scores achieved after listening to 'Healing Hearts' might actually reflect a relative improvement in the health knowledge of listeners. In fact, the broadcasts of 'Healing Hearts' on Alex FM were successful in reaching a disproportionate number of 25 - 44-year-old women ($\chi^2 = 17.3$, df = 2, $P = < 0.001$), although significantly fewer listeners lived in informal dwellings (51.9%) than non-listeners (66.3%; $\chi^2 = 4.82$, df = 1, $P = 0.006$). In view of these fundamental sociodemographic differences between listeners and non-listeners, it is surprising that there were no differences in health questionnaire scores between the two groups and it is possible that exposure to 'Healing Hearts' (and other Soul City media) had improved the (previously poorer) health knowledge of listeners.

Indeed, because a large number of listeners said that they enjoyed listening to 'Healing Hearts', and discussed its content with other people, it is clear that the broadcasts on Alex FM were successful in raising interest and awareness, both of which are important steps towards improving health behaviour.[3] Unfortunately, a variety of social and economic factors often limit the impact of interest and awareness on behaviour,[4] even when these subsequently lead to changes in knowledge and attitude. This might explain why Bardwell *et al*.[1] found a weaker relationship between maternal listenership and infant gastro-enteritis than between maternal listenership and 'good attitude'. Low-income mothers, such as those from Khayelitsha in their study, may be unable to implement health media messages,[5] even when these messages are effective at improving their attitude and knowledge.[1] For this reason it is perhaps naive to believe that media health campaigns always have a direct impact on health behaviour, although they can help to influence attitudes, knowledge and awareness, without which changes in behaviour are unlikely to occur.[3]

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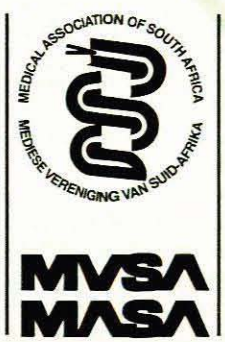

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Cost-effective on-site screening for anaemia in pregnancy

To the Editor: We read the article on 'Cost-effective on-site screening for anaemia in pregnancy in primary care clinics'[1] with interest. Even in developed countries, bedside testing is an important adjunct to laboratory-based diagnostic testing.[2] Where laboratory-based testing presents logistical problems, bedside testing is obviously more important.

The low specificity (81%; 95% confidence interval 77 - 85%) and poor positive predictive value (37%; 95% CI 29 - 47%) of the copper sulphate test as described by Wilkinson and Sach is of concern. When we evaluated the feasibility of the copper sulphate method to screen for anaemia in pregnancy,[3] we found a specificity of 95% (95% CI 88 - 98%) and positive predictive value of 80% (95% CI 56 - 93%). (In a Jamaican study a positive and negative predictive value of 100% was found![4]) Why the difference?

The poor specificity might have been caused by technical factors. If a copper sulphate solution with a higher specific gravity (SG) is used, the test should be more specific, possibly at the expense of sensitivity. Were other SGAEs investigated, or was the SG that we presented originally used[5] without further testing? Technical problems, such as temperature fluctuations, might also be more prevalent in a mobile clinic. Anyone interested in evaluating or implementing the copper sulphate test is encouraged to read the original articles on the subject.[6,7] These articles provide an exhaustive reference to technical causes of possible inaccuracies.

Another issue is the accuracy of the Sysmex analyser that was used as a 'gold standard'. How often and with what precision was the Sysmex analyser calibrated? Did the delay in performing the laboratory-based haemoglobin assay lead to inaccuracies?

Obviously, the human factor could also have played a part. The mental shift from the traditionally used haemoglobinometer estimation towards the copper sulphate solution is not always easy. A hand-held haemoglobinometer compensates for a lack of accuracy by providing the illusion of precision, whereas the copper sulphate test only provides a qualitative answer. Obviously, different copper sulphate solutions could be used to provide a semi-quantitive test.[3] When implementing the copper sulphate test in the antenatal clinic of Kalafong Hospital, we also found inaccuracies that were related to staff who were uncertain of the meaning of the symbols '>' and '<'. The staff now record the result as 'above 10' or 'below 10', and not as '> 10' or '< 10'.

This said, we would like to congratulate the authors on their drive towards bedside testing in antenatal clinics. They correctly remark that 'on-site testing . . . offers the opportunity to reduce unnecessary morbidity and mortality'.

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Dr Wilkinson replies: We are grateful for the interest in our work shown by Pistorius *et al*. We agree with the possible reasons given for the small differences in specificity and positive predictive value in our two studies. These observations affirm the importance of different groups' testing the same procedure in different settings, before reaching a firm conclusion about the utility of any intervention. Positive predictive value is strongly influenced by the prevalence of the condition being screened for. The Sysmex analyser was calibrated according to normal laboratory practice. In a pilot study we observed no difference between haemoglobin estimations made on the same sample between 0 and 72 hours after venepuncture, but thereafter estimation became highly variable; all specimens were therefore tested within 48 hours.

Growth, feeding practices and infections in black infants

To the Editor: The recent article on growth, feeding practices and infections in black infants by Delport *et al*.[1] highlights the confusion surrounding the use of National Centre for Health Statistics (NCHS) reference charts to assess the growth of children. Throughout the article these charts are described as 'standards' instead of 'references'. These charts were designed as *references*, for comparative purposes using samples or groups, and this use has been recommended by the World Health Organisation.[2] Indeed, Delport *et al*.[1] quite correctly emphasise that 'a distinction must be made between the concept of a reference and that of a standard or target'. However, their continued description of these references as 'standards', even though they use them appropriately as references, only perpetuates the conceptual confusion between these charts.

Delport *et al*.[1] also make the case for a local or national South African reference chart, as I have done on a previous occasion in this journal.[3] While abundant data are now available to create such a reference for South African urban and rural children, the eventual publication of such references is unlikely without a major shift in understanding and attitude by the paediatric community. The shift in understanding must be towards the realisation that references reflect the situation as it is, not necessarily as it should be. The attitudinal shift must be away from assuming that anyone who promotes the development of a national reference chart is advocating that the growth of South African children is good and that future interventions will not improve their growth, and thus health, status.[4] It is, of

course, understandable that during the apartheid era support for a chart that might be interpreted as indicating optimal growth, when in fact the growth of South African children was far from optimal, was thought to be misdirected. However, we are now in a post-apartheid era and such a national chart can only increase the sensitivity with which we can assess the growth of South African children.

The interpretation that the growth of breast-fed infants coincided with 'greater-than-average' growth performance with regard to weight gain must be viewed against the current debate as to the appropriateness of the NCHS references. Numerous studies of both breast-fed and bottle-fed infants[5-11] have highlighted the fact that samples in both developed and developing countries demonstrate an apparent acceleration in weight for age between birth and 6 months followed by a deceleration between 6 and 12 months. It is now internationally recognised that this so-called 'growth faltering' between 6 and 12 months is in fact a result of inadequacy of the NCHS reference data for the first 2 years of life.[5,12,13] That recognition has led to the WHO establishing a protocol for an international growth study in which participating mothers will exclusively breast-feed their offspring for 4 - 6 months, in accordance with WHO recommendations. The data resulting from these studies will be used to create truly 'international' growth references free from the current inadequacies of the NCHS charts.

Finally, I am concerned that Delport *et al.*[1] perpetuate the notion that height-for-age Z-scores less than the NCHS norms are 'probably due to a genetic influence'. It has been established unequivocally that differences in height for age are almost exclusively environmental in origin.[14] Delport *et al.*[1] describe their sample as being from 'urban or rural economically disadvantaged communities', which are characterised by poor maternal nutrition and health care in addition to deprived socio-economic circumstances and morbidity impacting on the infant postnatally. In such situations low height for age is the result of maternal and infant deprivation and is not the result of 'genetic influence'. Indeed, the genetic control of human growth does not begin to demonstrate its influence until well after the first year of life and during the first year environmental factors are of far greater importance in determining growth status.[15] To raise the spectre of a genetic difference in growth potential between groups of children in South Africa not only ignores the empirical evidence but also takes us back to a time in the recent history of this country when 'genetic differences' were used as a basis for discriminatory legislation.

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Dr Delport replies: Professor Cameron's constructive criticism is appreciated and accepted. His reiteration of the inadequacies of the NCHS reference data for the first 2 years of life is particularly relevant.

Geographical differences in infant feeding patterns in disadvantaged communities

To the Editor: The feeding patterns for infants born at Kalafong Hospital in Pretoria described by Delport *et al.*[1] provide an interesting comparison to the patterns observed in the Birth to Ten study among 1 267 children from similar disadvantaged communities in greater Soweto.[2] When these children were 6 and 12 months old, interviews with their principal caregiver provided data on infant feeding patterns similar to those described by Delport *et al.*,[1] including information on the duration of breast-feeding and the age at which non-breast-milk, cereal/porridge, maize meal and protein-rich supplementary foods (eggs, fish and/or meat) were introduced. In comparison with the infants studied by Delport *et al.*,[1] those from greater Soweto received very different diets (Tables I and II). Although a similar proportion (around 95%) of mothers in both studies breast-fed their infants for at least 1 month, from 6 months onwards significantly fewer of the mothers from greater Soweto continued to breast-feed. Not surprisingly, significantly more of these mothers made use of non-breast-milk feeds and significantly fewer were exclusively breast-feeding at 1, 3 and 6 months compared with mothers from the outskirts of Pretoria (Table I).

These geographical differences in infant feeding patterns illustrate how environmental and/or socio-economic differences influence infant diets even among similar, disadvantaged communities. To some extent these differences may reflect the socio-economic and/or social consequences of urbanisation, as 13.4% of the infants in Delport *et al.*'s[1] study were from a 'rural setting' while those from greater Soweto lived in established urban and peri-urban communities. Indeed, within the Birth to Ten study as a whole these mothers displayed the longest duration of breast-feeding, while those living in inner-city communities in central Johannesburg breast-fed on average for less than 4 months.[3]

The longer duration of breast-feeding among mothers from Delport *et al.*'s[1] study was accompanied by the use of

Table I. A comparison of milk intake among Birth to Ten infants from greater Soweto[2] and those from the outskirts of Pretoria examined by Delport *et al*.[1]

	Percentage of infants receiving					
	Breast-milk		Non-breast-milk		Exclusively breast-milk	
Age (mo.)	Greater Soweto	Pretoria east and west	Greater Soweto	Pretoria east and west	Greater Soweto	Pretoria east and west
1	94.4	94.6	16.7	3.6***	85.6	92.5*
3	85.2	89.6	46.9	8.3***	55.5	34.9***
6	73.7	93.3***	84.7	3.4***	0.8	7.2***
9	68.9	81.2*	85.4	14.1***	–	–
12	60.9	81.0***	85.4	15.2***	–	–

Significantly different from greater Soweto:
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

supplementary feeds comprising significantly less cereal and protein-rich foods, and significantly more maize meal, than the supplementary foods received by infants from greater Soweto (Table II). Mthimkhulu[4] described similar differences in the types of supplementary foods received by Birth to Ten infants from greater Soweto: those who were still being breast-fed at 6 months were significantly less likely to receive cereals ($P < 0.05$) or eggs ($P < 0.01$), and infants who were still breast-feeding at 12 months were significantly more likely to receive maize meal as a supplementary food

Table II. A comparison of supplementary foods received at 6 months by Birth to Ten infants from Diepmeadow and Soweto, and those from the outskirts of Pretoria examined by Delport *et al*.[1]

	Percentage of infants receiving		
	Cereal/ porridge	Maize meal	Eggs/ meat
Greater Soweto	87.6	57.2	60.0
Pretoria east and west	7.9***	91.0***	27.0***

Significantly different from greater Soweto:
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

($P < 0.05$) than those who had stopped breast-feeding. These results suggest that prolonged breast-feeding is often associated with a supplementary diet that contains little protein and little energy-dense food. Despite the acknowledged benefits of continuing to breast-feed for up to 2 years,[5] the association between prolonged breast-feeding (≥ 1 year) and a poor supplementary diet among mothers from disadvantaged communities might partly explain why a number of studies,[6] including Birth to Ten,[7] have found an association between prolonged breast-feeding and suboptimal growth. Similarly, this might partly explain why the children examined by Delport *et al*.[1] displayed relatively poor growth after 6 months of age when supplementary feeds play an increasingly important role in infant growth and development.[5] Interventions aimed at improving the growth of these children after 6 months of age should seek to protect breast-feeding while encouraging the use of more appropriate, nutrient-dense supplementary foods.

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Erratum

In the letter by Stein *et al*. entitled 'The Truth and Reconciliation Commission and post-traumatic stress disorder', which appeared on p. 763 of the June *SAMJ*, the names of two of the authors were misspelled. The correct spellings are Annamarie Schmidt and Alfred Allan.

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Tuberculous pleural effusions in HIV-positive patients

Non-reactive, multi-bacillary tuberculosis is associated with severe immune suppression and advanced HIV infection.[1] The small study by Trajman et al. (Int J Tuberc Lung Dis 1997; 1: 498–501) reporting on the histological parameters of pleural tuberculosis in HIV positive patients suggested that most HIV-positive patients with pleural TB have a preserved immune response resulting in pauci-bacillary TB with well formed granuloma. These results are in contrast with previous reports.[2,3]

We performed a prospective study of all patients (n = 74) with tuberculous pleural effusion admitted to Somerset Hospital, Cape Town, South Africa, during 1995 and 1996. Pleural histology was reviewed by a pathologist and classified as reactive (well formed granulomas), hypo-reactive (poorly formed granulomas) or non-reactive (no true granulomas), and scored for the number of acid fast bacilli (<1 or >1 per high powered field).[2]

HIV status was available for 66 patients. The HIV prevalence rate was 55%, which was higher than the 42% HIV prevalence of patients admitted with pulmonary TB during the same period. The median CD4 count in the HIV positive patients with pleural TB was 177/μL (range 4–760).

Adenosine deaminase (ADA) levels, suggestive of tuberculous aetiology if present in high concentration in the effusion,[4] were similar for HIV-positive and HIV-negative patients, irrespective of CD4 count (Table). Yield of pleural culture for *Mycobacterium tuberculosis* tended to be increased in patients with advanced immune suppression. Histological evaluation revealed that hypo-reactive pattern occurred in all groups, and multi-bacillary TB was unusual, even in patients with low CD4 counts (Table).

Tuberculous pleural effusions in HIV-infected patients are associated with moderately advanced HIV disease, as evidenced by CD4 count[5] and survival pattern.[6] In agreement with the findings of Trajman et al., histological grade and number of acid-fast bacilli on pleural biopsy were similar for HIV-negative and -positive patients. It is suggested that TB pleural effusions in HIV-positive patients occur prior to the onset of major defects in cell-mediated immunity.

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Table

	HIV− (n = 30)	HIV+ CD4 >200 (n = 16)	HIV+ CD4 <200 (n = 20)
Effusion ADA* median	102	114	92†
MTB culture positive	14/26 (54%)	7/15 (47%)	12/16 (75%)†
Histology			
Reactive	13/18 (72%)	6/14 (43%)	4/8 (50%)†
Hypo-reactive	5/18 (28%)	8/14 (57%)	4/8 (50%)†
Acid-fast bacilli on biopsy			
<1/hpf	16/17 (94%)	9/9 (100%)	6/7 (86%)†
>1/hpf	1/17 (6%)	0/9	1/7 (14%)†

*adenosine deaminase.
†$P > 0.05$ for comparison HIV−, HIV+ CD4 > 200 and HIV + CD4 < 200.
ADA = adenosine deaminase; MTB = *Mycobacterium tuberculosis*; hpf = high powered field.

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Severe arthropathy with ofloxacin in two cases of MDR tuberculosis

The 4-quinolone group of drugs is now being used extensively for various infections, including tuberculosis, due to their efficacy and very few side effects. In immature animals they are reported to have produced arthropathy of weight-bearing joints.[1] Pefloxacin and other 4-quinolone derivatives are reported to produce arthropathy, tendinitis and tendon rupture in humans,[2–4] but there are no reports so far regarding arthropathy attributed to ofloxacin. This drug is now being used extensively in MDR tubercu-

losis as a second-line drug, along with other drugs such as pyrazinamide, which is known to produce arthropathy due to hyoperuricemia.[5] When arthropathy develops in such situations, it is difficult to attribute it to either pyrazinamide or ofloxacin, hence it is essential to distinguish pyrazinamide-induced arthropathy from the ofloxacin-induced condition.

We report our experience of similar situations when two patients with MDR tuberculosis developed arthropathy after receiving ofloxacin with other antituberculosis drugs in the course of therapy. Case I: A 30-year-old female south Indian doctor attended our OPD with cervical lymphadenitis. Biopsy of the gland suggested tuberculosis etiology. She had taken antituberculosis treatment (ATT) with 2 months of ethambutol (E) 800 mg, INH (H) 300 mg, rifampicin (R) 450 mg and pyrazinamide (Z) 1500 mg OD, followed by 4 months of INH 300 mg and rifampicin 450 mg OD (2EHRZ/4HR) irregularly before attending our OPD. Since the patient gave a history of irregular ATT, she was started on a regimen of 2SHRZ + ofloxacin (Ofl) 200 mg BD, clarithromycin (CL) 500 mg OD and cycloserine (C) 250 mg BD with 10 HRE and ofloxocin. [Regimen-1, 2 SHERZ + Ofl + C+ CL and 10 HRE + Ofl] Her biochemical parameters, including serum uric acid level, were normal. The patient noticed swelling and joint pain with severe tenderness and local rise of temperature around both knees and ankle joints after 5 days of treatment. There was also tenderness on the plantar aspect of the right foot which was aggravated by the movements of the toes. With these features, a clinical diagnosis of arthritis of the ankle and knee, with tendinitis of the right foot was made. X-rays of both joints were normal. Since pyrazinamide is known to produce hyperuricemia and arthritis,[5] it was withdrawn and the serum uric level was analysed. The patient was continued with other drugs along with anti-inflammatory drugs. [Regimen-II, SHER + Ofl + CL, C). There was no improvement in her condition after 1 week of treatment; on the contrary, she became completely bedridden. Her uric acid level was 4 mg/dl, within the normal limits. Since 4-quinolones are reported to produce arthropathy, ofloxacin was withdrawn (Regimen-III, SHERZ + C + CL) and within 4 days there was a dramatic improvement in her condition. In order to confirm whether ofloxacin had actually produced arthropathy or not, the drug was reintroduced at the same dosage, along with other drugs (i.e., Regimen I was given). The patient again developed arthropathy within 2 days thus confirming ofloxacin as the offending drug. Ofloxacin was withdrawn from the regimen and the patient continued on other drugs. She was followed up for one year on 2SHERZ + C + CL and 10 HRE (Regimen III). The pain and swelling in the knees and ankle joints completely subsided. X-ray of the joints was normal, but she continued to have pain in the right foot, probably due to tendinitis, for a long time after the withdrawal of ofloxacin. Her lymph nodes completely regressed after one year of treatment.

Case II: A 35-year-old south Indian nurse had developed tuberculous adenitis and had taken regular ATT with INH, rifampicin and ethambutol for one year, but the gland did not regress and repeat biopsy suggested TB adenitis. The culture from the gland yielded MDR tuberculosis. The patient was put on 2 EHRZ + ethionomide 500 mg (ET) OD, ofloxacin (Ofl) 200 mg BD, and cycloserine 250 mg (C) BD. Her blood count, lives function tests (LFT) and uric acid level were normal before the start of treatment. On the 6th day of treatment she developed severe pain in the knee joints. Her uric acid level was again normal. Pyrizinamide was withdrawn but there was no improvement, then ofloxacin was withdrawn and patient had a dramatic relief from the pain. Treatment was continued with the other drugs along with pyrazinamide, and the patient had no further symptoms of joint pain. Her lymph glands completely regressed.

Quinolones as a group, and pefloxacin in particular, are known to produce cartilage toxicity in animals and human beings.[3] So it is reasonable to speculate that ofloxacin, being a quinolone, could have caused cartilage toxicity and arthropathy. It is difficult to ascertain whether or not synovial inflammation was the cause of arthropathy, as there was no effusion in our patient. The presence of tendinitis in case No. 1 raises a number of questions. Was there any drug binding to the macromolecules in the joints and tendons, inciting inflammatory reaction? Was it due to chronic inflammatory reaction in the tendon that the patient experienced tendinitis even long after withdrawal of the drug? If so, the long term effects of ofloxacin treatment, which is given particularly in MDR tuberculosis, needs to be analysed in case control studies. Ofloxacin in normal therapeutic doses is usually eliminated completely within 24 to 36 hours. Hence the renal mechanism does not appear to be the cause of its toxicity when given alone; however when it is given along with other drugs such as cycloserine in the treatment of MDR tuberculosis, there may be an impairment of glomerular clearance of ofloxacin, leading to elevated serum levels causing toxicity. This aspect needs to be studied further in assays of serum ofloxacin levels during the course of MDR tuberculosis treatment.

Pyrazinamide-induced arthropathy should be distinguished from that of ofloxacin, as both drugs are given concomitantly to treat MDR tuberculosis. Pyrazinamide produces arthropathy by inducing hyperuricemia. The urate crystals are usually precipitated in synovial fluid and induce inflammatory reaction. The diagnosis is usually made by detecting high serum uric acid levels and demonstrating urate crystals in synovial fluid.

The role of MDR tuberculosis in producing ofloxocin-induced arthropathy, and ethnic differences in its interreaction with other drugs, need to be studied further as arthropathy has not been reported in any Western literature.

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DOTS and beyond: towards a holistic approach to the conquest of tuberculosis

The excellent, thought-provoking editorial by John Grange[1] on this subject brings out many points of great relevance to the management of tuberculosis, especially in the developing countries. The World Health Organization (WHO)[2] has been campaigning in strong terms that Directly Observed Treatment, Short-course (DOTS) is the most important 'breakthrough' in the treatment of tuberculosis that has been achieved in recent times. WHO[3] has stated that DOTS 'is the name for a comprehensive strategy by which health workers counsel and observe their patients swallowing each dose of a powerful combination of medicines and the health services monitor the patients' progress until each is cured.' For successful outcome of the DOTS strategy,[3] WHO has highlighted five essential elements: prompt diagnosis, direct observation of the ingestion of the drugs, proper record keeping, adequate and constant supply of the drugs and the necessary funding to implement this program. In no unambiguous terms, it has stressed that 'political and financial commitment and a dependable drug supply are essential parts of the DOTS strategy'.

It is clear, as has been pointed out by Grange,[1] that the DOTS philosophy rests solely on the premise that the patient is totally responsible for the failure of chemotherapy. This assumption is not completely correct in the vast majority of situations prevailing in the developing countries,[4] though it may be more applicable in developed countries. In fact Banerji[5] has estimated that the patient's role in the failure of chemotherapy in developing countries due to noncompliance can constitute less than 10% of the total causes. The noncompliant patient in the US, as has been excellently described by Reichman,[6] typically fits into the picture demanding DOT. On the other hand, the behaviour of the non-compliant patient in developing countries is quite frequently not because of his innate nature or irresponsible conduct of non-co-operation, but as an unfortunate consequence of the drug delivery and treatment systems, which, as Grange[1] has pointed out, have many shortcomings. The typical non-compliant patient in such countries, as was described by Pamra[7] some years ago, is the one who is forced to ignore or, at the most, give a lower priority to his tuberculosis treatment, than to his other demanding responsibilities—job, wages and even food and shelter. As Grange[1] and Gangadharam[8] have mentioned, many, if not all, of the so-called non-compliant patients in developing countries would be compliant, if a proper drug delivery system were functioning. For instance, if the clinics are made to operate to suit the convenience of the patients, with minimum turnaround time, and, more importantly, if there is an uninterrupted supply of all the prescribed drugs, there should definitely be an excellent compliance on the part of the patients in these countries.

Even when the drugs are provided, and the dispensaries are scheduled to operate under conditions which are most suitable for the vast majority of patients, as suggested by Grange[1] and Pamra,[7] there is another unrecognised serious problem of corruption.[8] In many countries, such malpractices are prevalent. Even though the drugs are purchased by the hospital pharmacies, they are channelled through corrupt practices to 'black market' dispensaries. DOT can correct this only when the infrastructure itself is fully competent and honest. Besides proper procurement of all the drugs, a problem which Grange[1] has alluded to, proper and honest distribution should be ensured. As has been mentioned earlier,[8] every member in the hierarchy of drug delivery—directors, doctors, nurses, pharmacists and field workers—should be conscientiously devoted to his or her assigned duties. Only then will any method, be it DOT or another method of drug delivery, function properly and produce the expected results. What we need in many developing countries is DOD (Directly Observed Doctors) as Olle-Goig[9] has indicated, or more appropriately, DOP (Directly Observed Program or Directly Observed Provider), not simply DOT or DOTS!

Based on all these, it is correct to state, as Grange[1] implied, that DOT or DOTS is not a universal recipe for controlling tuberculosis. Iseman et al.[10] have stated that it is a procedure which cannot be ignored. Their recommendations are perhaps applicable to countries like the US, where the patient is mostly to be blamed, and funds and administrative machinery are available to the best possible extent. The application of DOT has indeed 'turned the tide' in an otherwise trying situation as existed in New York.[11] On the other hand, whenever the availability of necessary funds and facilities, particularly adequate supply of drugs, are wanting, less than optimal results will be obtained as has been cautioned by Riechman.[12] Finally, it is worth emphasizing another important fact which was unfortunately missed by Grange.[1] DOT or DOTS was born more than 34 years ago in Madras, India.[13] In fact, the picture of a nurse directly observing a patient taking the pills, a DOT scenario, which the WHO has put in one of its pamphlets,[14] is from that original classical Madras study.[13]

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Editor's note

Professor Gangadharam correctly points out several issues which confound the DOTS strategy. However, his assessment of drug-taking behavior in patients in developing countries may be overly optimistic. Study after study documents substantial nonadherence with diverse treatments in persons from all racial, ethnic, national, economic, social and educational groups. Much as we anticipate certain patterns of clinical manifestations with *Mycobacterium tuberculosis*, so might we regard nonadherence as a predictable attribute of *Homo sapiens*.

Registration and treatment of patients with smear-positive pulmonary tuberculosis

Quarterly reports from district tuberculosis (TB) officers on case-finding and treatment outcome are an essential component of the monitoring system of a National Tuberculosis Control Programme. It is recommended by the International Union against Tuberculosis and Lung Disease (IUATLD) that all patients recorded as smear-positive in the laboratory register be compared with the list of patients commenced on treatment in the TB register, to ensure that no patients identified in the laboratory go without treatment.[1] It is further recommended by the World Health Organization that smear-positive patients in the laboratory register who have never appeared for registration or treatment must be registered in the TB register and should be classified as treatment interrupted (defaulters) in the treatment outcome.[2]

In practice, these recommendations are often not followed, at least in Malawi, which is said to have a well-functioning TB Control Programme. Although the laboratory register is compared with the TB register during routine supervisory visits, quarterly reports are based solely on assessment of the TB registers. We decided to 1) document the proportion of smear-positive TB patients in the laboratory register who do not appear in the TB register and the possible reasons, and 2) the length of time between laboratory diagnosis and commencement of treatment of smear-positive patients during country-wide visits in 1997.

There are 43 central, district or mission hospitals in Malawi that perform sputum smear microscopy for acid-fast bacilli in PTB suspects and which then register smear-positive PTB patients for treatment. During the first 6 months of 1997, 41 hospitals (3 central, 22 district and 16 mission) were visited by the TB Programme Management Group, and TB officers were requested to record, during the next 6 months, 1) all new patients registered in the hospital labora-

tory sputum register as smear-positive, and 2) whether these smear-positive patients had been registered in the hospital TB register and commenced on short-course chemotherapy. The dates of these entries and activities were noted. If patients were not registered in the TB register, TB officers were asked to find out wherever possible what had happened.

Data on 3482 smear-positive PTB patients in laboratory registers from 41 hospitals were collected over a mean period of 5.4 months (range 2–8 months); 2980 (86%) patients were registered in the TB register. The length of time between diagnosis in the laboratory register and commencement of treatment was documented for 2975 patients; it was 7 days or less in 2534 (85%) patients, 8–14 days in 311 (10%) patients, 15–21 days in 76 (3%) patients and 22–88 days in 54 (2%) patients. 502 (14%) patients were not registered in the TB register. Of 595 patients with smear-positive PTB in the three central hospitals, 236 (40%) were not registered (range 34%–44%). Of 991 patients with smear-positive PTB in the 16 mission hospitals, 107 (11%) were not registered (range 0–36%). Of 1896 smear-positive PTB patients in the 22 district hospitals, 159 were not registered (range 0–17%). The reasons for non-registration in all 41 hospitals are shown in the Table.

This study shows that 86% of patients in the laboratory register were registered and placed on anti-tuberculosis treatment, and that in 85% of registered patients the time between laboratory diagnosis and start of treatment was one week or less. Although we did not document reasons for the delays in starting treatment, this is often because of patients submitting sputum specimens at health centres and the logistic difficulties encountered in getting results back to the patient and getting the patient to report at the hospital for registration and treatment. A significant proportion (14%) of smear-positive PTB patients failed to be registered in the hospital at which the sputum smear diagnosis was made. The proportion of failed registrations was particularly high in the three central hospitals; this is disappointing, given that a reasonably successful cough register system was set up in one of the central hospitals in 1995.[3] In one quarter of all patients there was a documented reason for failed registration such as early death or registration and treatment at another district in the country. Although in the majority of patients the reasons were not known, we suspect that many registration failures can be explained by reasons documented in the Table.

As a result of this study and other operational research studies focusing on diagnostic issues and treatment outcomes, we have started two initiatives to understand and to try and reduce the problem. First, all TB officers will be required on a regular basis to check the laboratory register and indicate in writing, against all smear-positive patients, the management outcome (i.e., either the TB treatment registration number or another outcome such as death/transfer to another district). This will be monitored every quarter by regional TB officers using a structured check list, and we have set a target for the National TB Programme that 95% of smear-positive PTB patients will be registered and placed on treatment. Second, in each region there is now a quarterly meeting of TB officers so that they can compare and share their information on transfer out and default rates; we hope that this type of peer-review will contribute to improved performance. The aspect of TB control is of importance because at present Malawi is under-reporting both its true smear-positive TB case rate and its true default rate.

Table Resasons for smear-positive PTB patients in the laboratory register not being registered in the hospital TB register

Reasons for not being registered	*n* (%)
Died before registration	40 (8%)
Registered and treated in another district in Malawi	27 (5%)
Absconded and not traced	20 (4%)
Transferred to another district in Malawi: registration and treatment status unknown	18 (4%)
Transferred to Mozambique: registration and treatment status unknown	5 (1%)
Discharged from hospital before smear results known and not traced	4 (1%)
Refused to be treated*	2
Reason unknown	386 (77%)
Total	502

*One of these patients believed he had been bewitched and left to seek the services of a traditional healer.

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Tuberculin PPD RT23 is stable: quality control data cannot be denied

We have read the rebuttal[1] of Dr. Kim and his colleagues to our counterpoint paper "Tuberculin PPD RT23: still going strong"[2] with interest, and have noted their arguments that RT23 was indeed used in most of the surveys and that BCG vaccination may not have changed tuberculin reactivity in TB patients.

However, the rebuttal does not deal adequately with the quality control data in our paper. It is stated that "quality control in animal models is not possible with low doses such as 1 T U directly and it is very true that the smaller the dose, the greater the variation in potency." This statement is clearly wrong. RT23 dilutions prepared at Statens Serum Institut are controlled according to the European Pharmacopoeia,[3] which describes a potency assay for 1 and 2 T U dilutions. This monograph further requires that the observed potency must be in the interval 80–125% of the stated potency and that "The fiducial limits (P = 0.95) of error are not less than 64% and not more than 156% of the stated potency." These requirements to the precision of the potency determination of 1 and 2 T U dilutions are precisely the same as for more concentrated dilutions of tuberculin.

The rebuttal therefore does not present any arguments for the statement[1] that "The SSI cannot claim that there was no potency change with the quality control data observed in guinea pigs." Indeed, the data given in Figures 1, 2 and 3 of the counterpoint paper[2] do not give any indications of a potency loss of RT23. Finally, we wish to emphasise the point given in our paper[2] that if the decline in tuberculin reactivity seen in Korean TB patients should be explained by a loss of potency of RT23, it can be calculated that RT23 should have lost more than 90% of its activity in the period 1965–1995! This fact, which is not commented on in the rebuttal of Dr. Kim and colleagues, is clearly incompatible with both the quality control data in guinea pigs as well as with the recent clinical study in Mexico showing equivalent reactivity of 2 T U of RT23 and 5 T U of Tubersol.[2]

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CORRESPONDENCE

Unrecognised *Mycobacterium tuberculosis*

Sir—Clifford McDonald and colleagues (Oct 2, p 1159)[1] report data from 344 febrile inpatients in Malawi and Thailand who had mycobacterial blood cultures and HIV testing in addition to routine care. Their findings confirm those of a study from Abidjan, Côte d'Ivoire,[2] in which all patients with mycobacteraemia had advanced HIV infection, as indicated by a mean CD4-cell count of 49/μL and mortality of 80%. However, whether scarce resources should be used for expensive investigations on patients with a poor outcome is debatable.

We are concerned that McDonald and colleagues' study promotes the increased use of mycobacterial cultures when much cheaper tests—ie, repeat sputum smears and wide-needle aspirate of lymph nodes—would suffice. We have done a study of 141 consecutive HIV-positive inpatients with confirmed tuberculosis to assess yield and timeliness of results.[3] Blood culture was by far the least cost-effective diagnostic approach, whereas the most cost-effective was lymph-node aspirate. Results of 16 of 19 positive blood cultures were returned after the diagnosis had been made by other means. Our findings are in keeping with those of others.[4]

It should also be noted that many sick patients find it difficult to produce sputum on the day of admission and on-the-spot samples have a lower yield than those collected overnight. In our series only 17 (31%) of 54 patients diagnosed on sputum smear were identified on the day of admission. Unrecognised tuberculosis may therefore be an artifact created by McDonald and colleagues' case definition.

Finally, a search for unrecognised *Mycobacterium tuberculosis* would be best done by sputum culture (higher yield and lower unit cost). Use of blood cultures would be rational if the original intention were to look for unrecognised *M avium-intracellulare*. No cases seem to have been found, in accord with other findings.[5]

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Sir—Clifford McDonald and colleagues[1] report that 10% of febrile adults admitted to hospitals in Malawi and Thailand had mycobacteraemia at the time of admission. However, the conclusions drawn from this study have surprised some of us working in Malawi. The data from Malawi are presented as though these patients with unrecognised *M tuberculosis* bacteraemia would not have been correctly diagnosed during their hospital stay.

The constellation of chronic fever, cough, or weight loss (not including oral thrush), picked out by the investigators as predicting myco-bacteraemia, would routinely trigger clinicians in Malawi to investigate for tuberculosis with sputum-smear microscopy and radiography.[2] Unfortunately, the investigators give no follow-up data on the outcome of the patients, many of whom may have been correctly diagnosed and treated during their admission. In Malawi, these patients are likely to have been started on tuberculosis treatment before the results of blood cultures became available. How often did the results of blood cultures in McDonald and colleagues' study influence patient management?

The researchers raise concerns about the nosocomial risk posed by the admission of undiagnosed tuberculosis cases to medical wards, especially where rates of HIV infection are high. However, that they chose to use mycobacteraemia in febrile patients to argue that blood cultures are required to identify infectious cases, and therefore to reduce the risk of nosocomial transmission, is surprising. Surely the cause of concern should be the 113 (33%) of 343 patients who had chronic cough in addition to fever, and who may have been expectorating viable mycobacteria in their sputum.

We collected blood samples from 113 patients with chronic cough with axillary temperatures greater than 37·4°C. Tuberculosis was confirmed in 55 patients (48·5%): 33 were sputum smear positive and 22 were smear negative but culture positive. 11 patients had tuberculosis isolated from blood cultures (ten had *M tuberculosis* and one had atypical mycobacteria). All patients with mycobacteraemia were HIV positive. Eight of these patients had acid-fast bacilli detected in their sputum. The remaining three patients had chest radiographs consistent with pulmonary tuberculosis, and were started on tuberculosis therapy before the results of blood cultures were known. Pulmonary tuberculosis was confirmed by sputum smear or culture in a further 44 patients for whom blood-culture samples were negative. Blood culture did not contribute to management of patients in any of these cases, and sputum-smear microscopy rapidly identified patients constituting the greatest nosocomial risk.

We suggest that a better strategy for preventing nosocomial transmission would be rapid (same-day) acid-fast bacilli smear microscopy for patients admitted with chronic cough.

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Diagnosing HIV-associated tuberculosis: reducing costs and diagnostic delay

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SUMMARY

SETTING: University-affiliated hospital in South Africa.
OBJECTIVE: To assess the time to diagnosis and the yield and laboratory cost of diagnostic procedures in human immunodeficiency virus (HIV) associated tuberculosis.
DESIGN: Cohort study.
PATIENTS: Adult HIV-infected patients with newly-diagnosed tuberculosis admitted over a 2-year period.
RESULTS: A total of 141 admissions fulfilled the case definition. Sputum smear yield (43% overall) correlated strongly with chest radiograph appearance but not with CD4+ lymphocyte count. Sputum smear yield was approximately 40% per sample sent, resulting in a high cumulative yield when ⩾ three samples were sent. Smear of sputum or wide needle lymph node aspirates were the most cost-effective diagnostic methods. Significant diagnostic delay occurred in sputum smear-negative patients. Most patients with sputum smear-negative tuberculosis had either pleural effusions or lymphadenopathy. Lymph node biopsy had a high diagnostic yield even in patients with symmetrical nodes, but was under-utilised in this group. There was unnecessary expenditure on cultures, with many patients having several positive cultures.
CONCLUSION: Repeated sputum smear examination produces a high cumulative yield in HIV-associated tuberculosis. Considerable savings in laboratory utilisation and bed occupancy would have been made if a streamlined diagnostic approach with greater use of lymph node aspirate and early pleural or lymph node biopsy had been followed.
KEYWORDS: HIV infection; tuberculosis diagnosis; sputum smear; needle aspirate; South Africa.

TUBERCULOSIS IS the leading cause of mortality and morbidity in human immunodeficiency virus (HIV) infected patients in Africa and Asia.[1–4] In both developing[5] and industrialised[6] nations, delayed or missed diagnoses of tuberculosis have important adverse consequences including nosocomial transmission, death, and wasted bed occupancy.[5,6] Sputum smear is the preferred method of rapid diagnosis of tuberculosis due to its low cost. However, most[7–9] but not all[10,11] studies of HIV-infected patients with tuberculosis have found a higher rate of smear-negative sputum.

The aims of the present study were to investigate the clinical correlates of smear-negative sputum in HIV-infected in-patients with tuberculosis, to assess recent recommendations to reduce the number of sputum smears from three to two per patient-episode,[12] to examine resource utilisation in cases where sputum is smear-negative, and to propose a cost-effective approach to rapid diagnosis.

METHODS

The study was conducted in an urban university-affiliated hospital providing general in-patient services over the period October 1994 to September 1996. During this period all patients with suspected tuberculosis were offered HIV testing and 88% accepted. During the study period the policy was to send at least one sputum specimen for mycobacterial culture for all HIV seropositive admissions. Mycobacterial culture was encouraged on biopsy material as well as other specimens. Discharge summaries of patients treated for tuberculosis were collected prospectively and cross-checked against the laboratory database and monthly records of HIV-positive admissions compiled by the hospital infection control nurse to ensure that all patients with proven or empirically treated tuberculosis were identified. Radiological and clinical data were abstracted from the medical records and the laboratory database was used to identify investigations for tuberculosis.

The case definition for tuberculosis required one of the following three criteria: 1) culture of *Mycobacterium tuberculosis* from any site; 2) an anatomical diagnosis of tuberculosis at necropsy; 3) the presence of acid-fast bacilli (AFB) on a smear or granulomas on histology with subsequent response to treatment. Patients who defaulted from treatment and presented

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with early relapse were excluded. Sputum smear-negative tuberculosis was defined as negative smear on all sputum samples sent, irrespective of the number of specimens sent. Smears of sputum and aspirates from lymph nodes were examined using the fluorochrome method except when urgent processing was requested, in which case Ziehl-Neelsen staining was done. Mycobacterial cultures were performed with a radiometric system (BACTEC™). Laboratory cost data were obtained from the March 1996 unit costs supplied by the laboratory. The unit costs include reagents, staff and other costs.

Fisher's exact test and χ^2 with Yate's continuity correction were used for comparison of proportions as appropriate. The Kruskall-Wallis test was used for comparison of means.

RESULTS

During the review period there were 415 medical admissions of HIV-infected patients, of whom 179 were treated for tuberculosis. Mycobacterial culture of sputum was requested for 51/69 patients with respiratory conditions other than tuberculosis, and for 157/179 patients treated for tuberculosis. Of 149 admissions that fulfilled the case definition of tuberculosis, eight patients who had defaulted and presented with early relapses were excluded as the diagnosis was already available, leaving 141 admissions in 131 patients (10 patients had recurrent tuberculosis). HIV was acquired by heterosexual transmission in 122/131 (93%) patients and by homosexual transmission in the remainder. There were 91 Africans, 30 Coloureds (a mixed race community) and 10 Caucasians. CD4+ lymphocyte counts were done within a month of admission in 103 patients (median count 83/μl, interquartile range 37–207). In the 141 admissions the diagnosis was confirmed by culture of *M. tuberculosis* in 109 (77%), a treatment response coupled with AFB on histology or sputum smear in 21, granulomas on histology together with a treatment response in six, and on necropsy in five.

Diagnostic yield of sputum

Sputum smear was positive in 60 admissions (43%), negative in 49 and sputum could not be obtained in 32. Sputum smear was more likely to be positive if the clinical presentation suggested pulmonary involvement (cough, chest pain or focal chest signs): 59/124 (48%) had clinical evidence of pulmonary involvement compared with 1/17 (6%) who did not ($P < 0.01$). Detailed description of the chest radiograph was available for 91 of the 124 patients with clinical evidence of pulmonary involvement—the radiographic appearance correlated with the yield of sputum smear (Table 1). The yield of sputum smear was not related to the CD4+ lymphocyte count: 35/68 (51%) with pulmonary symptoms and a CD4+ lymphocyte count <200/μl were smear-positive compared with 10/25 (40%) with higher counts (odds ratio 1.59, 95% confidence interval 0.57–4.48, $P = 0.45$).

Table 1 Chest radiographic appearance and yield of sputum smear (χ^2 for linear trend 13.08, $P < 0.01$)

Radiographic pattern	Smear-negative (or no sputum)	Smear-positive *n* (%)
Pleural effusion	11	1 (8)
Normal	12	5 (29)
Nodular infiltrate	22	14 (39)
Consolidation	7	15 (68)
Cavitation	0	4 (100)

In view of recommendations to reduce the number of sputum smears,[12] we examined the yield of smears of consecutive sputum specimens. Sputum smear yield was not significantly different for consecutive samples (45/109 [41%] for the first, 20/61 [33%] for the second, 12/32 [38%] for the third and 8/18 [44%] for the fourth; χ^2 for linear trend 0.027, $P = 0.868$). The incremental yield from repeating the smear on those patients negative on preceding smears was 8/39 for a second, 4/21 for a third and 3/12 for a fourth specimen. The yield of smear on first sputum was 17/29 (59%) in patients able to produce a sample on the day of admission compared to 22/65 (34%) when sputum was obtained after day one ($P = 0.04$).

The yield of culture of the first sputum sample was 25/34 (74%) on smear-negative patients and 37/43 (86%) on smear-positive patients ($P = 0.28$). Thirty-three patients had two sputum samples cultured with a similar yield (26/33 and 28/33), but five patients who were negative on the first specimen were positive on the second.

Resource utilisation

To reflect clinical decision-making, time to diagnosis was assessed for admissions where the diagnosis was based on specimens obtained during admission, i.e., excluding necropsy diagnoses (five patients) and diagnoses made on specimens sent from the clinic prior to admission (8 patients). Mean time to diagnosis was 13 days when sputum was smear-negative or the patient could not produce sputum, compared to 2 days for smear-positive tuberculosis ($P < 0.01$). Of smear-negative admissions, mean time to diagnosis was 11 days for patients who had a biopsy compared to 20 days for patients who did not ($P = 0.01$). For smear-negative admissions with a biopsy diagnosis, mean time to diagnosis was 3 days in patients undergoing pleural biopsy compared to 7 days for patients undergoing biopsies of other tissues ($P = 0.04$).

In patients with smear-negative sputum or no sputum, wide needle aspirate and/or biopsy was performed in 13/43 (30%) with symmetrical lymph node enlargement compared to 13/17 (76%) with lymph

nodes noted to be asymmetric in size ($P < 0.01$). Patients with negative aspirate went on to biopsy only when the nodes were noted to be asymmetric. This difference in use of investigations occurred despite no difference in yield: 6/7 biopsies of symmetrical node enlargement were positive compared to 8/10 for biopsies of asymmetrical nodes, and the corresponding figures for aspirates were 3/7 and 5/9.

Table 2 displays for each investigation the diagnostic yield and cost per diagnosis. Due to the large numbers of positive results obtained after a previous positive result, diagnostic yield is shown both as number of positive results and number of occasions when the positive result was the first evidence of tuberculosis. Cost per diagnosis is calculated as total cost (number of investigations multiplied by the March 1996 unit costs) divided by the number of occasions when the positive result was the first evidence of tuberculosis. One diagnosis was made on culture of a skin biopsy[13] but, as this was the only investigation of this type, it is excluded from Table 2 along with five cases diagnosed at autopsy and eight on samples sent prior to admission.

Given the high cost per diagnosis of many investigations and frequent delay in obtaining diagnoses, we modelled laboratory utilisation using the diagnostic yield data from the 127 admissions in Table 2. Sputum smear-negative patients fell into three groups—those with pleural effusions, those with lymph nodes >1 cm and those with neither. Investigations for patients with pleural effusions could be limited to histology of pleural biopsy followed by trial of therapy pending results of pleural fluid culture. The laboratory cost for the 22 patients with pleural effusion would have decreased from US$1015 to US$570 (44% reduction), but three patients would have been empirically treated instead of confirmed as tuberculosis. Investigations for patients with lymph nodes >1 cm can be limited to wide-needle aspirate followed by excision biopsy if the aspirate smear is negative. The laboratory cost for the 40 sputum smear-negative patients with nodes >1 cm would have decreased from US$1615 to US$445 (72% reduction), but four patients (10%) would have required further investigations to confirm the diagnosis of tuberculosis. Only 10 patients were smear-negative or unable to produce sputum and had neither effusion nor nodes >1 cm.

Table 2 Laboratory cost per diagnosis (calculated as the total cost for each investigation divided by the number of occasions when the positive result was the first diagnostic result)

Specimen type	Yield (%)	First diagnostic result	Cost per diagnosis (US$)
Sputum			
Smear	85/220 (39)	54	$9
Culture	116/142 (82)	17	$78
Lymph node			
Aspirate	8/16 (50)	8	$5
Biopsy	14/17 (82)	13	$20
Pleura			
Histology	15/21 (71)	15	$20
Culture	6/9 (67)	1	$82
Pleural fluid			
Smear	2/20 (10)	2	$26
Culture	12/20 (60)	4	$45
Bone marrow			
Histology	5/19 (26)	3	$103
Culture	6/16 (38)	2	$67
Urine			
Smear	1/26 (4)	1	$67
Culture	11/26 (42)	3	$74
Liver			
Histology	3/4 (75)	1	$112*
Blood			
Culture	19/45 (42)	3	$267

* Includes cost of liver function tests and tests of coagulation function.

DISCUSSION

Tuberculosis in patients co-infected with HIV is associated with more extra-pulmonary disease and, as immunity declines, more non-cavitating pulmonary tuberculosis.[14] This presents diagnostic difficulties as sputum is often unobtainable or smear-negative. This is reflected in the present study, where 23% of patients were unable to produce sputum and the overall yield of sputum smear was 43%. As we have shown, negative sputum smears are associated with diagnostic delays. The most important resource used in the diagnosis of tuberculosis is bed occupancy, and the time to diagnosis is critical.[15] A more rapid diagnosis would also reduce morbidity and mortality in HIV-infected patients.[6]

The yield of sputum smear in our study was approximately 40% per sample sent. We believe this is an accurate reflection of HIV-associated tuberculosis in in-patients, as a large number of sputum samples were assessed and the diagnosis of tuberculosis was unlikely to be missed in our extensively investigated cohort. The incremental yield of sputum smear depends on the yield per sample sent. If this is 80%, then >95% of cases who are sputum-smear positive will be detected on two smears (80% positive on the first smear and 80% positive on the remaining 20%—i.e., 96%). This was the finding of an outpatient study in Tanzania of patients of unknown HIV status.[12] That study has influenced policy to the extent that the South African National Tuberculosis Programme requires only two sputum samples be sent from clinics. In our in-patient HIV cohort with a sputum smear yield of 40% per sample sent, six specimens would need to be sent in order to detect >95% of cases. However, it would waste bed occupancy to wait for six sputum samples before embarking on other tests. With a yield of 40% per sample, three smears will detect almost 80% of cases—it would thus be reasonable to proceed to invasive diagnostic

tests after three negative sputum smears. Further smears can still be sent, but the additional yield will be progressively lower and no more than six in total should be sent.

The observed lack of relation of CD4+ lymphocyte count to sputum smear result is in keeping with one previous study by Smith et al.[11] but contrasts with another by Jones et al.,[16] and requires further study. This was a surprising finding given that there was a good correlation of the CD4+ lymphocyte count with the chest radiographic appearance, which, as we have previously shown, correlates well with the CD4+ lymphocyte count.[14]

Based on our finding that most patients with sputum smear-negative tuberculosis have either pleural effusions or lymphadenopathy, we suggest a streamlined diagnostic approach which would result in considerable savings in laboratory utilisation and bed occupancy (Figure). Early pleural biopsy and aspiration should be done in patients with pleural effusions. The combined yield of these two procedures is very high. Most clinicians would be comfortable continuing anti-tuberculosis treatment even if histology and cultures are negative if there is a satisfactory response. Lymphadenopathy is significantly associated with tuberculosis in HIV-infected patients presenting with respiratory disease.[17] Lymph node aspirates have a high yield in HIV-associated tuberculosis.[18,19] and were under-utilised in our cohort. This was the most cost-effective diagnostic test. Our finding that lymph node biopsy was often diagnostic even when the nodes were symmetrically enlarged, in contrast with tuberculous lymphadenitis in HIV seronegative patients, is in agreement with a large central African study performed by Bem.[20] Provided that the clinical picture suggests tuberculosis, clinicians should not hesitate to biopsy nodes that are symmetrically enlarged. Despite the aggressive approach to diagnosis in our patients, three patients died without treatment and without investigation, all with symmetrical lymph node enlargement.

Patients who are sputum smear-negative and have neither effusion nor lymph nodes (only 8% in this study), together with those with a negative lymph node biopsy, could have a trial of therapy pending culture results if the clinical picture is strongly suggestive of tuberculosis. The specimen with the highest culture yield in our study was sputum, whilst urine, blood and bone marrow had a similar yield. Culture of bone marrow aspirate or blood should be performed wherever atypical mycobacteria are common.[21,22] The yield of the two investigations is similar, but the bone marrow trephine biopsy provides an opportunity to make a rapid diagnosis if granulomas or AFB are found (26% yield in the present study). Blood culture was an expensive diagnostic test in our study largely because it was slow and other cultures were often positive first. In addition, a reasonable yield of blood cultures can only be assured by case selection on CD4+ lymphocyte count (modest yield with counts 100–200 $\times$ 10^6/l and high yield with counts under 100),[16] adding to expense. Urine culture is valuable even in the absence of pyuria.[23] Sending two or three specimens for mycobacterial culture prior to a trial of therapy should be sufficient—reducing the number of specimens cultured has been identified by others as an important area for cost savings.[24]

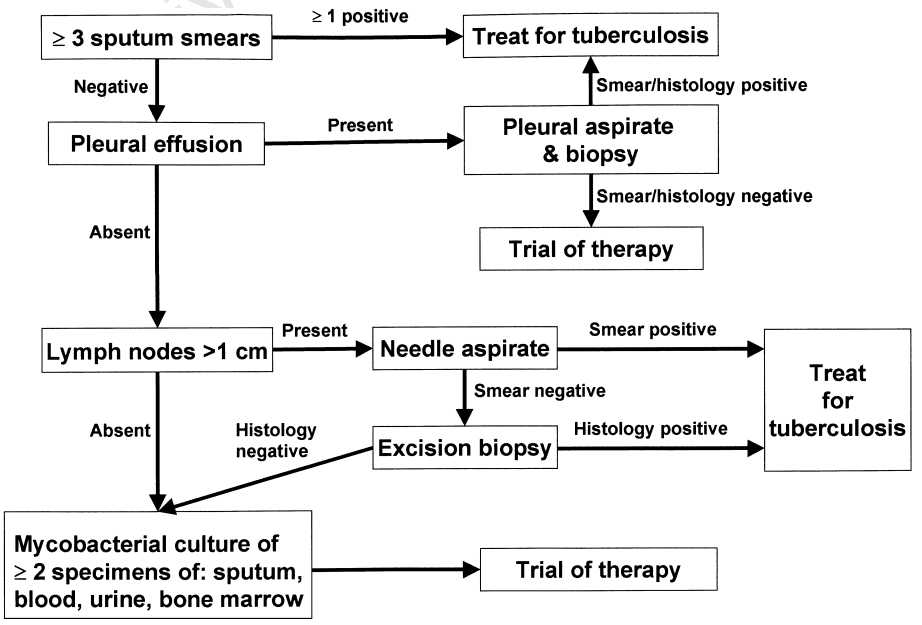

Figure Proposed diagnostic algorithm for HIV-infected patients with suspected tuberculosis. Trial of therapy should only be undertaken if other diagnoses are excluded or unlikely.

We believe that our findings are relevant to settings with different resource constraints from ours. In more resource-rich settings, more cultures could be sent and procedures such as bronchoscopy could be used. We obtained diagnostic confirmation of tuberculosis without resorting to bronchoscopy (which was difficult to arrange during the study period); this is in keeping with the finding of Daley et al. that bronchoscopy is of little value in areas where tuberculosis is endemic.[25] In more resource-poor settings, where mycobacterial cultures are often unavailable, the early use of lymph node aspirate followed by biopsy or pleural aspirate and biopsy would yield the diagnosis in most cases. In resource-poor settings algorithms for empirical treatment based on symptoms can be used for the remaining cases.[17]

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RÉSUMÉ

CADRE : Hôpital d'Afrique du Sud affilié à l'Université.
OBJECTIF : Déterminer la durée nécessaire au diagnostic, le rendement et le coût en laboratoire des procédés de diagnostic dans la tuberculose associée au virus de l'immunodéficience humaine (VIH).
SCHÉMA : Etude de cohorte.
PATIENTS : Sujets adultes infectés par le VIH et admis au cours d'une période de deux ans pour une tuberculose récemment diagnostiquée.
RÉSULTATS : 141 admissions ont répondu à la définition des cas. Le rendement du frottis d'expectoration (globalement 43%) est en étroite collaboration avec l'aspect du cliché thoracique, mais non avec les décomptes de lymphocytes CD4+. Le rendement du frottis d'expectoration fut d'environ 40% par échantillon, ce qui entraîne un rendement cumulatif élevé de l'envoi de trois échantillons ou davantage. Les méthodes de diagnostic dont le rapport coût-efficacité est le meilleur sont le frottis d'expectoration ou la ponction-aspiration ganglionnaire au moyen d'une grosse aiguille. Un délai significatif de diagnostic existe chez les patients dont l'expectoration est négative à la bacilloscopie. La plupart des patients atteints d'une tuberculose à bacilloscopie négative dans l'expectoration souffraient soit d'épanchements pleu-

raux, soit d'adénopathies. La biopsie ganglionnaire a un rendement diagnostic élevé même chez les patients dont les adénopathies sont symétriques, mais elle a été sous-utilisée dans cette étude. L'on a fait des dépenses inutiles pour des cultures chez beaucoup de patients dont plusieurs cultures se sont avérées positives.

CONCLUSION : La répétition de frottis d'expectoration entraîne un rendement cumulatif élevé dans les tuberculoses associées au VIH. D'importantes économies en matière d'utilisation du laboratoire et d'occupation des lits auraient pu être faites si une approche de diagnostic rationnelle avait été suivie, faisant appel aux ponctions ganglionnaires et aux biopsies pleurales ou ganglionnaires précoces.

RESUMEN

MARCO DE REFERENCIA : Hospital afiliado a la Universidad de Sud África.

OBJETIVO : Evaluar el tiempo de diagnóstico, el rendimiento y el costo de laboratorio de los métodos de diagnóstico en la tuberculosis asociada con VIH.

MÉTODO : Estudio de cohorte.

PACIENTES : Pacientes adultos infectados por el VIH con tuberculosis reciente, hospitalizados en un período de dos años.

RESULTADOS : En total, 141 pacientes hospitalizados cumplieron los requisitos del objetivo. El rendimiento del examen de esputos (43% del total) se relacionaba fuertemente con las imágenes radiográficas pero no con el recuento sérico de linfocitos TCD4. El rendimiento del examen de esputo fue aproximadamente del 40% por cada muestra enviada y aumentó fuertemente con el envío de $\geq$3 muestras. El examen de esputos o la aspiración con aguja de los ganglios fueron los métodos de más alto rendimiento costo-beneficio. Existió una demora diagnóstica significativa en los pacientes con esputo negativo. La mayoría de los pacientes con tuberculosis y esputo negativo tenían una pleuresía o adenopatías. La biopsia ganglionar tuvo un gran rendimiento aún en pacientes con ganglios simétricos, pero fue poco utilizada en este grupo. Existió un gasto innecesario con los cultivos.

CONCLUSIÓN : Los análisis repetidos de esputos producen un alto rendimiento acumulativo en la tuberculosis asociada al SIDA. Se hubieran logrado ahorros considerables en el uso del laboratorio y ocupación de las camas si se hubiera recurrido más a la aspiración ganglionar, a la aspiración pleural temprana o a la biopsia ganglionar.

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Risk Factors for Developing Tuberculosis in HIV-1–Infected Adults From Communities With a Low or Very High Incidence of Tuberculosis

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Objective: To estimate the incidence rate of tuberculosis in HIV-1–infected adults resident in a region with a high tuberculosis prevalence and to identify clinical and laboratory parameters associated with increased risk of developing tuberculosis.

Methods: Adult patients going to the University of Cape Town HIV clinics between January 1986 and May 1996. The following variables were assessed for the risk of developing tuberculosis: ethnicity, employment and education status, World Health Organization (WHO) clinical stage, erythrocyte sedimentation rate (ESR), $CD4^+$ count, and total lymphocyte count. Tuberculin skin test data were not available.

Results: There were 198 prevalent and 144 incident cases of tuberculosis in the cohort of 1206 patients. The incidence rate of tuberculosis risk was 10.4/100 person years. WHO clinical stages 3 and 4 (risk ratio [RR], 3.4; 95% confidence interval [CI], 1.8–6.4), ESR >75 mm/hour (RR, 3.5; CI, 1.8–6.5) and being a member of a high-prevalence tuberculosis community (RR, 2.5; CI, 1.2–5.1) were independently associated with the risk of developing tuberculosis.

Conclusions: HIV-infected adults in Cape Town are at high risk of developing tuberculosis irrespective of tuberculin skin testing. The risk increases markedly with HIV disease progression. Patients at extremely high risk can be identified on the basis of demographic and clinical features. Such individuals would be suitable for targeted tuberculosis prophylaxis.

Key Words: HIV-1—Tuberculosis, epidemiology—South Africa—CD4 lymphocytes—Erythrocyte sedimentation rate.

An estimated 1.7 billion people, many of whom live in sub-Saharan Africa, are latently infected with *Mycobacterium tuberculosis* (1). The spread of the HIV epidemic in Africa has resulted in large numbers of dually infected individuals and increasing tuberculosis (TB) incidence rates in sub-Saharan African countries (2,3).

TB has been a disease the diagnosis of which has required physicians to notify government authorities in South Africa since 1921, with the highest such notification rates being reported in Cape Town and the Western Cape region. Before onset of the present heterosexual HIV epidemic, this region had one of the highest reported TB incidence rates in the world, at 670/100,000 per annum (4,5). The four main communities of the Western Cape in descending order of population size are "coloured" (a term used in South Africa to describe a mixed race group in South Africa and used here with that spelling to denote this particular group within the South African context the paper describes), African, white, and Asian. TB rates in the coloured and African communities are extremely high, while rates in whites and Asians are similar to those found in industrialized countries (4,5).

During the period of this study, adult HIV seropreva-

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lence increased rapidly, initially in the homosexual population (almost exclusively white and coloured patients) and subsequently in the heterosexual population (predominantly African) (6). An unlinked anonymous antenatal survey in 1994 estimated adult HIV seroprevalence in the region to be 1.18% (7). Adult HIV seroprevalence is increasing most rapidly in the growing periurban informal settlements of Cape Town, where antenatal HIV seroprevalence reached 7% in 1995 (Professor Moodie, personal communication, 1996). The increasing HIV epidemic in a population with such a high TB prevalence poses a major challenge to the existing TB control program. TB prophylaxis of dually infected individuals has recently been shown to be effective in Africa (8), but this is difficult to implement because of the large numbers of patients at risk and operational difficulties (9).

The aim of this study was to estimate the incidence rate of TB in HIV-infected adults from communities with both low and extremely high prevalence of TB, and to assess prognostic markers that would allow identification of patients at particularly high risk. Such groups may be suitable for targeted TB prophylaxis.

METHODS

The study population consisted of adult patients going to the HIV outpatient clinics of Somerest and Groote Schuur hospitals, Cape Town, between January 1986 and May 1996. Both hospitals are teaching institutions of the University of Cape Town and were the only public sector HIV clinics in the region for most of the study period; thus there could be no significant referral bias. The same protocols were used at both clinics and there was partial sharing of clinical staff. HIV-1 infection was confirmed by two enzyme-linked immunosorbent assays (ELISAs) on two nonsimultaneous blood samples. Patients were evaluated according to their clinical condition: those with early disease were seen at 3- to 6-month intervals, while advanced patients were seen monthly or more frequently if necessary.

Patients were clinically staged at each visit, using the World Health Organization (WHO) clinical staging system (10). In this system, clinical stages 1 and 2 represent early disease, stage 3 is similar to the AIDS-related complex, and stage 4 is AIDS. The performance scale (a symptom score) was not used for staging purposes. WHO clinical staging was done retrospectively before December 1991 by two independent observers with good interobserver agreement ($\kappa = 0.91$) and prospectively after that date. Patients presenting with TB at initial visit were excluded from subsequent cumulative risk analysis. TB was diagnosed by passive case-finding. The case definitions for TB were: definite culture of *M. tuberculosis* or autopsy diagnosis, probable smear positive or granulomata on histology (with or without caseation or smear positivity), and possible clinical response to drug therapy (short-course rifampicin-based regimens). Cases diagnosed by clinical response alone were only included after careful review (by Wood); radiologic response was required for patients with abnormal chest radiographs. Patients diagnosed in this way typically presented with intrathoracic adenopathy or exudative lymphocytic pleural effusions with negative cultures. Pulmonary TB was defined as a pulmonary infiltrate, or sputum smear or culture positivity, or a pleural effusion. Extrapulmonary TB was defined as disease not confined to pleura or lung and included disease affecting other intrathoracic structures such as mediastinal lymphadenopathy. Isoniazid prophylaxis was not routinely prescribed during the study period.

TB-free survival of patients (excluding TB prevalent cases) was assessed by Kaplan-Meier analysis. Patients were censored at last visit date and at death. The predictive value of ethnic group, employment status, highest education level, gender, WHO clinical stage 1 to 4, CD4 T-cell count, total lymphocyte count, and erythrocyte sedimentation rate (ESR) for subsequent TB-free survival was further investigated by Cox-proportional hazards modeling using a stepwise selection procedure (PHEG procedure, SAS software, SAS, Cary, NC, U.S.A.). Transformations were used for CD4 and lymphocyte counts (logarithmic) and ESR (square root).

RESULTS

The patient cohort consisted of 1206 adults infected with HIV-1. There were 342 cases of TB (198 prevalent and 144 incident cases): 38% definite, 48% probable, and 14% possible. Pulmonary TB was present in 66% and extrapulmonary in 34%. $CD4^+$ T-lymphocyte counts at the time of TB diagnosis were available for 195 patients and ranged from 1 to 823 cells/μl with a median of 113 cells/μl. The median $CD4^+$ T-lymphocyte count at time of pulmonary TB diagnosis was 159 cells/μl and for extrapulmonary TB it was 90 cells/μl. Sixty five percent of all TB and 85% of extrapulmonary TB occurred in patients with less than 200 $CD4^+$ T-lymphocyte/μl.

Demographic data for the 1,008 patients who did not have TB at initial visit is shown in Table 1. There were

TABLE 1. *Demographic characteristics of the 1008 patients who did not have tuberculosis at initial assessment.*

Gender	
Male	65%
Female	35%
Mean age (yr)	32 ± 9
Sexual preference	
Homosexual	36%
Heterosexual	64%
Ethnic group	
White	31%
Coloured	24%
African	43%
Asian	1%
Clinical stage	
WHO 1	48%
WHO 2	17%
WHO 3	25%
WHO 4	10%
CD4+ lymphocyte count (cells/μl)	
<200	34%
200–400	34%
>400	32%
Mean ESR (mm/hour)	44 ± 41

Percentages do not always sum to 100 due to rounding

WHO, World Health Organization of the United Nations; ESR, erythrocyte sedimentation rate; SD, standard deviation.

1391 total person years of observation, 232 (23%) died, and 313 (31%) were lost to follow up (defined as not having been seen by physicians at the clinics for >12 months). The incidence rate of TB was 10.4/100 person-years. The incidence rate of TB by race for patients with both early and late HIV disease together with the risk ratios compared with those found in the general population of Cape Town (11) are shown in Table 2.

Ethnic grouping was a strong confounder of TB-free survival and was included in all subsequent modeling. TB-free survival did not differ significantly between African and coloured patients ($p = .10$) who were then combined for further analysis as the high-prevalence TB community. Similarly, there were no significant differences in TB-free survival between WHO clinical stages 1 and 2 ($p = .69$) and clinical stages 3 and 4 ($p = .62$), so that these categories were also combined for further analysis. There was a significantly higher ($p < .001$) risk of developing TB in advanced (WHO stages 3 and 4) versus early (WHO stages 1 and 2) HIV disease for both high and low prevalence TB communities (Fig. 1).

WHO clinical stage was the most predictive variable for the development of TB with ESR second, $CD4^+$ T-lymphocyte count third, and total lymphocyte count fourth. Of 520 patients with complete information on all the variables at first visit, 54 (11%) developed TB with censored data from 466 (89%) of patients. Exploratory analysis of TB events versus ESR revealed that the incidence was stable <75 mm/hour but increased at higher values. The predicted TB-free survival in clinical stage 3/4 patients with ESR <75 mm/hour was similar to the survival profile of clinical stage 1/2 patients with ESR >75 mm/hour. ESR >75 mm/hour remained strongly predictive of the subsequent development of TB after excluding patients who developed active TB within 3 months of measuring ESR (risk ratio [RR], 3.62; 95% confidence interval [CI], 1.57–8.37).

Cox-proportional hazard modeling, demonstrated that only ethnic group, clinical staging, and ESR contributed significant independent information to the modeling of TB-free survival. We found no differences in these findings when data were analyzed separately for the periods before and after December 1991 to assess the effect of retrospectively assigning WHO clinical staging, but the risk of developing TB was higher after December 1991 as a result of the increasing proportion of patients from high-prevalence TB communities. $CD4^+$ lymphocyte count was not significantly associated with the risk of developing TB after adjustment (RR, 1.6; 95% CI, 0.9–2.7). Employment status and education level were not independently associated with TB-free survival. The estimated risk ratios of a final multiple Cox regression model using WHO clinical stage (3 and 4), ESR (>74 mm/hour), and high-prevalence TB community with male gender as a control are shown in Table 3.

DISCUSSION

The high incidence rate (IR) of TB reported in this study is the result of the development of an HIV epidemic in a population with one of the highest TB prevalence rates in the world. The overall TB IR in the Cape Town HIV-infected population is similar to that reported in defined high-risk HIV cohorts, such as intravenous drug users in Madrid at 9.1/100 person-years (12), New York at 9.8/100 person-years (13), and patients in Haiti at 7.5/100 person-years (14). The RR for TB associated with HIV infection in African and coloured patients with WHO stage 1 and 2 disease is similar to that reported for HIV-positive individuals elsewhere in Africa. HIV-associated RRs of 4.7, 8.3, and 10 have been reported from the Ivory Coast (15), Tanzania (16), and Zaire (17), respectively. The high prevalence of TB in coloured and African communities is a reflection of their poor socioeconomic circumstances (5). Socioeconomic indicators available for analysis in the study were relatively crude and did not include measures defining crowding or total household income. Although the highest IRs were in African and coloured patients, it is of interest that the highest RR were recorded in white patients. One possible

TABLE 2. *Race-specific tuberculosis incidence rates for HIV-infected people stratified by World Health Organization (WHO) clinical stage*

	Tuberculosis incidence/100 person years (95% CI)		Risk ratio[a]	
Race	WHO 1 and 2	WHO 3 and 4	WHO 1 and 2	WHO 3 and 4
White	3.5 (1.8–5.2)	7.6 (4–11.1)	140	304
Coloured	5.0 (2.7–7.3)	31.8 (19.9–43.6)	10.1	64.4
African	8.7 (5.3–12.1)	68.5 (52.7–84.3)	10.3	81.5

[a] Risk ratios were calculated using the general Cape Town, South Africa, population incidence (11); HIV incidence rate (per 100 person years)/population incidence (per 100,000 per year (10^{-3}).

Data is not shown for Asians due to their small numbers.

CI, 95% confidence intervals.

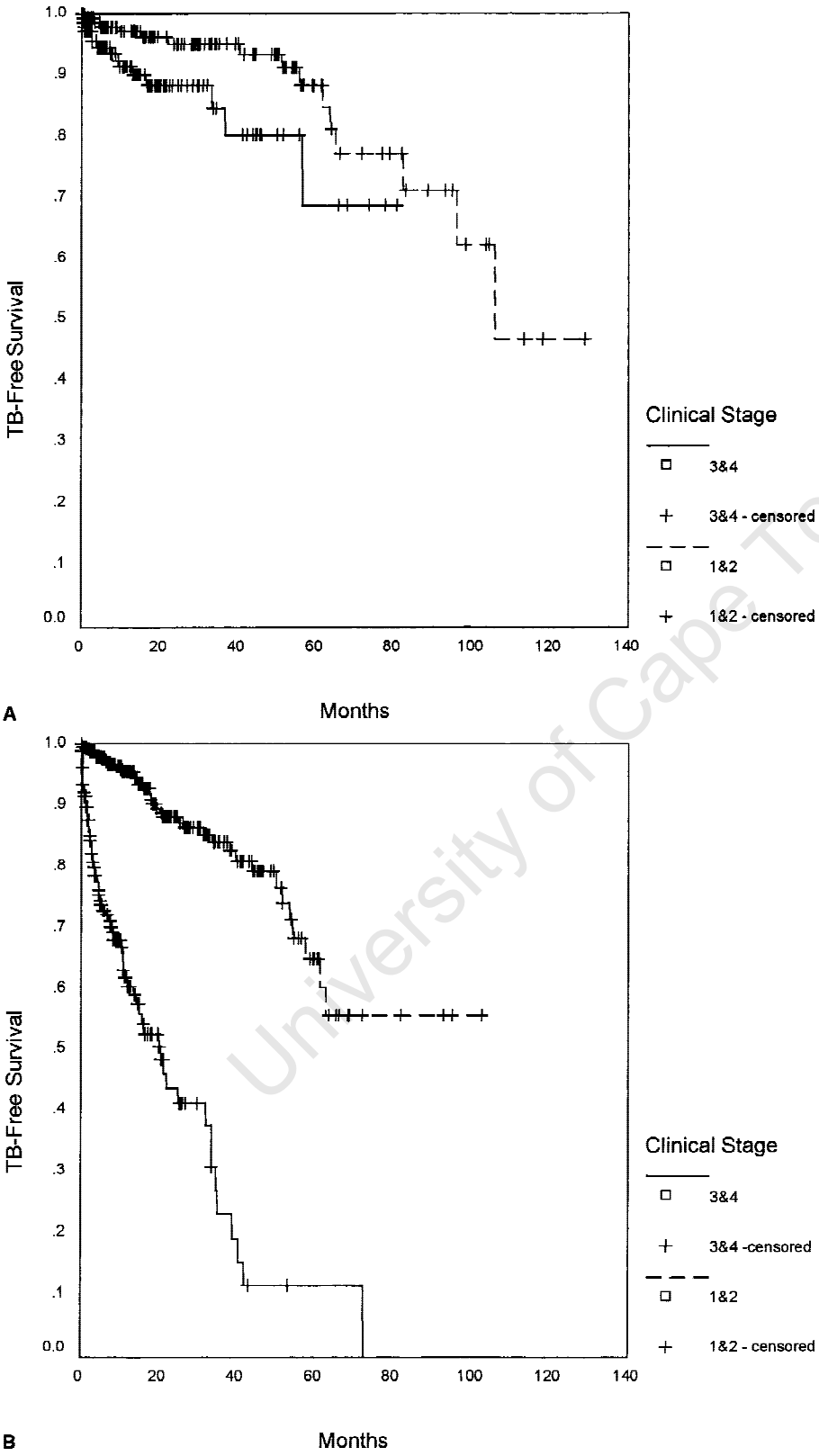

FIG. 1. Infected patients with World Health Organization stage 1/2 or stage 3/4 disease from (**A**) low-prevalence (white and Asian) and (*B*) high-prevalence (African and coloured) tuberculosis communities. Risk of tuberculosis was significantly higher in those with advanced HIV disease ($p < .001$).

TABLE 3. *Adjusted risk ratios with 95% confidence intervals (CI) derived from Cox proportional hazard modeling of predictors for tuberculosis*

	Risk ratio (95% CI)	*p* Value
WHO stages 3 and 4	3.4 (1.8–6.4)	.0001
ESR >75 mm/hr	3.5 (1.8–6.5)	.0001
High-prevalence TB community	2.5 (1.2–5.1)	.0122
Male gender	1.4 (0.7–2.6)	.3155

African and coloured patients are from high-prevalence tuberculosis communities.

WHO, World Health Organization of the United Nations; ESR, erythrocyte sedimentaion rate; TB, tuberculosis.

explanation for this is that white patients could have been exposed to *M. tuberculosis* in our clinic waiting areas.

A major finding of this study was that the risk of developing TB increased markedly with advancing HIV disease. The resultant IRs in WHO stage 3 and 4 disease exceeded the reported prevalence of positive tuberculin skin reactivity in these communities (18), which may reflect a high rate of acquisition of new TB infections in patients without prior exposure. A weakness of the present study is the absence of tuberculin skin test data. The predictive value of tuberculin skin testing, however, is limited in a population such as that of Cape Town with a high immunization rate with bacille Calmette-Guérin (5). Furthermore, anergy is likely to be common in patients with WHO stage 3 and 4 disease whose annual risk of TB, in African and coloured patients, exceeded 30% in the present study. We have found Mantoux induration of >5 mm in only 24% of African and coloured patients with WHO stage 3 and 4 disease in an ongoing isoniazid prophylaxis study.

Although previous studies in Africa have shown a strong association between HIV infection and TB, they have not demonstrated a marked increase in TB incidence with HIV disease progression. Two studies from Central and West Africa have reported higher $CD4^+$ T-lymphocyte counts of patients with TB and HIV-infection than in the present study (19,20). However, both studies were limited to sputum smear–positive pulmonary TB and would have underdiagnosed extrapulmonary TB as well as the more atypical pulmonary presentations associated with advanced immune suppression (21). In contrast, autopsy studies from Zaire and the Côte d'Ivoire showed evidence of disseminated TB in 41% and 54% of AIDS patients, respectively (22,23) with a median $CD4^+$ T-lymphocyte counts of 88 cells/μl in the Zaire study (22). The facilities for histology and TB culture available in our hospitals may have increased case finding, because radiographic presentation of pulmonary TB in our HIV-infected patients is frequently atypical (21) and 52% of cases provided a negative result on sputum smear. A recent prospective multicenter U.S. cohort study (24) found the risk of developing TB was significantly higher in patients with a $CD4^+$ T-lymphocyte count <200 cells/μl.

The finding that WHO clinical staging was a more powerful prognostic risk factor for TB than the $CD4^+$ T-lymphocyte count is of particular importance in Africa, where flow cytometry and $CD4^+$ T-lymphocyte counts are not generally available. The association of high ESR with increased subsequent progression to TB parallels the observation that ESR is a predictor of mortality in HIV infection (25,26).

Prophylaxis has been shown to effectively reduce TB in tuberculin skin test–positive HIV-infected patients in Haiti (14) and Uganda (8). Tuberculin skin testing is operationally difficult in resource-poor settings (9) and, as already indicated, there is a high rate of anergy in patients with advanced disease who are at highest risk. Furthermore, the duration of benefit after TB prophylaxis is short-lived (27,28). Life-long or repeated TB prophylaxis has been suggested, but this would cause major operational problems. However, life-long prophylaxis may be feasible for patients with advanced HIV infection in high-prevalence TB communities because their survival is limited (access to antiretroviral therapy is limited in most areas where TB is highly prevalent). Cohorts of HIV-infected patients at very high risk for TB can be identified by simple clinical HIV staging. Our data indicate that the risk is high irrespective of tuberculin skin test status. Patients with advanced HIV disease have either been excluded from trials of TB preventive therapy (8,14) or were only a small proportion of the study population (28), thus indicating the need for further studies in these patients.

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SOUTHERN AFRICAN HIV CLINICIANS SOCIETY

AIDS REVIEW

DOES ANTIRETROVIRAL THERAPY HAVE A ROLE TO PLAY IN THE CONTROL OF TUBERCULOSIS IN SOUTH AFRICA?

Tuberculosis is the most common opportunistic infection occurring in HIV infection in Africa.[1] This is especially so in South Africa, where one of the worst TB epidemics in the world, with disease rates more than double those observed in other developing countries and up to 60 times higher than those currently seen in the USA or Western Europe, is raging. There is great variation among the South African provinces, and the worst hit is the Western Cape, with incidence rates of approximately 600/100 000 reported in 1996. These numbers have been projected to increase considerably with the HIV epidemic unless effective control is achieved, and will result in an estimated 3.5 million new cases of TB by 2005 and a staggering 90 000 deaths - all due to a treatable and fully curable illness[2] (Fig. 1).

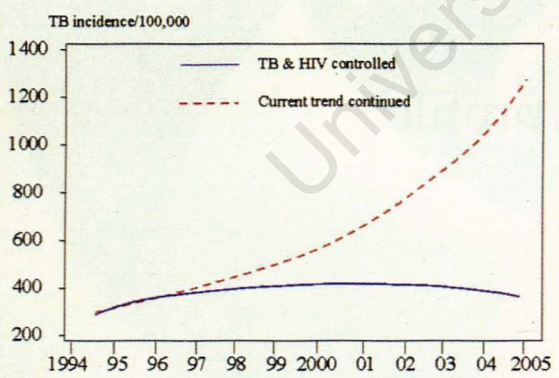


Fig. 1. Predicted tuberculosis incidence rates/100 000 population at current rates of increase and modelling to show what can be achieved if the HIV/TB epidemic were controlled.

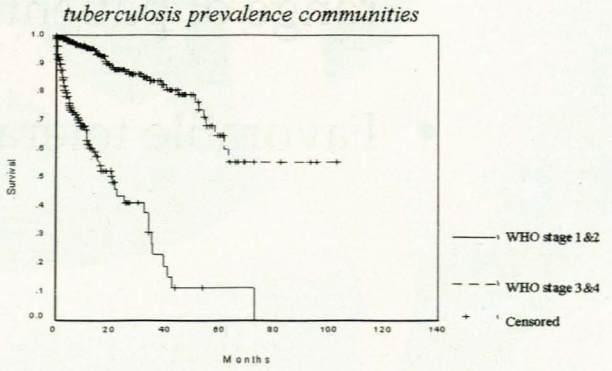
IMPACT OF HIV ON THE TB EPIDEMIC

It is clear that this epidemic must have a severe public health impact as numbers of cases of HIV-associated TB increase and overwhelm TB programmes and primary care health services. For example, TB incidence rates per 100 000 population in a high-prevalence reporting district in Cape Town increased from 804 in 1997 to 957 in the first quarter of 2000.[3] We have learnt many lessons from the Hlabisa area in KwaZulu-Natal, where the extremely high HIV rates have had an adverse impact on TB and the TB programme in the district.[4] The current strategy of the national TB control programme, based on positive sputum smears, passive surveillance and emphasis on improving treatment completion rates, is failing to control this HIV-driven TB epidemic.

IMPACT OF HIV ON THE INDIVIDUAL WITH TB

Patients infected with HIV are at increased risk of both reactivation and new-infection TB, particularly as their immunity declines with advancing HIV infection. In a large group of patients with advanced HIV from a high TB prevalence area in Cape Town the median time to development of TB was 20 months (with incidence rates of 31.8 - 68.5/100 per year of follow-up)[5] (Fig. 2). In addition, we know that the diagnosis of TB has serious survival implications for these patients. It has been shown in HIV-infected patients in Cape Town that a diagnosis of TB was associated with an increased risk of death at all CD4+ T-cell counts.[6] Tuberculosis results in an increase in HIV replication and elevated viral loads months after the TB is diagnosed and the patient otherwise appears to be responding favourably to TB treatment.[7,8]

816 HIV-infected adults living in high tuberculosis prevalence communities



R.Wood, *et al. AIDS* 2000; 23:75-80

Fig. 2. Kaplan-Meier plots of tuberculosis-free survival for HIV-infected patients with WHO stage 1 and 2 or 3 and 4 from high-prevalence (black and coloured) tuberculosis communities. Risk was significantly higher in advanced HIV disease ($P < 0.001$).

It is clear that if we are to prevent this morbidity and mortality on an individual as well as a community basis some new and specific strategies need to be considered. While increased resources should certainly go into further improving and strengthening TB programmes, it has been shown through mathematical modelling that this will be insufficient on its own.[9] Strategies such as active case-finding, recommended by the World Health Organisation in their report on the global

HIV/AIDS epidemic in June 2000,[10] as well as INH prophylaxis in HIV-infected individuals, are modalities that will not be discussed further here. A further strategy on which little has been written is the use of antiretroviral therapy (ART) to reduce the incidence and impact of tuberculosis.

IMPACT OF ART ON TB EPIDEMICS IN THE WORLD

ART has been shown to reduce the incidence of TB in HIV-positive patients in developed countries. In Italy it has been noted that there has been a decline in cases of TB following the introduction of highly active ART (HAART).[11] In a large cohort of HIV patients from 11 US cities where the TB incidence was 5 cases/1 000 years, the risk of TB was much lower among those taking HAART (relative risk (RR) 0.2, 95% confidence interval 0.1 - 0.5) than among those not prescribed HAART .[12] Owing to lack of access to HAART in developing countries where TB rates are considerably higher, the efficacy of ART in preventing TB in these settings has not been established. Recent data from Cape Town have just become available, and the following very encouraging findings have been reported.[13]

In this study, 378 patients on antiretroviral therapy attending a University of Cape Town HIV clinic from 1996 to 2001 were compared with 562 similar control patients not on ART and attending the same clinic from 1992 to 1996. The patients had advanced disease. Those treated had median baseline CD4 T-cell counts of 244 and median $\log^{10}$ viral loads of 5.4, and 40% of patients were at World Health Organisation clinical stages 3 or 4. Adherence to ART in this group was remarkably high — 92% at 48 weeks.

Using a strict case definition of tuberculosis, TB incidence was 9.9/100 per year in the untreated control group and decreased to 2.1/100 per year in the triple therapy group, despite similar background TB rates in the population over both periods.

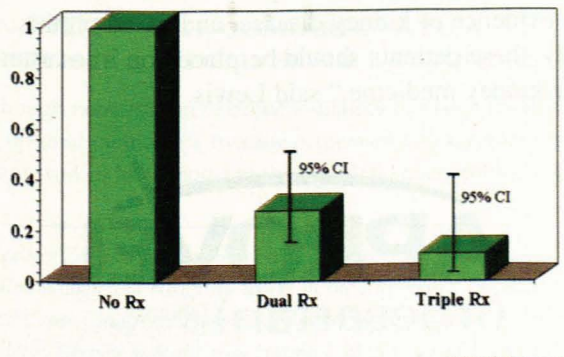


Fig. 3. Adjusted relative risk for developing tuberculosis in an HIV-infected Cape Town cohort on dual or triple antiretroviral agents compared with those not on antiretroviral treatment.

With Cox proportional hazards modelling adjusting for age, clinical stage, baseline CD4 level and ethnicity, the adjusted RR for dual (some patients had been on two antiretroviral agents only) and triple therapy was 0.28 and 0.11 respectively, which was highly significant (Fig. 3).

Predictive variables in the triple therapy arm were baseline CD4+ T-cell count of less than 200 (adjusted RR 2.6) and clinical stages 3 and 4 (adjusted RR 2.7), while for each log increase in baseline viral load the adjusted RR increased by 3.1. Reduced adherence to antiretroviral therapy (< 90% of tablets taken over the study period) was strongly associated with development of tuberculosis in these patients (adjusted RR 122).

These data show that antiretroviral therapy significantly decreases the incidence rate of tuberculosis, even in a setting where TB rates are among the highest in the world, and may be a powerful strategy for the control of HIV-associated tuberculosis even when programmes and health services are swamped with the huge increases in TB rates that come with the HIV epidemic.

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Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study

Motasim Badri, Douglas Wilson, Robin Wood

Summary

Background Studies of the effect of highly active antiretroviral therapy (HAART) on the risk of HIV-1-associated tuberculosis have had variable results. We set out to determine the effect of HAART on the risk of tuberculosis in South Africa.

Methods We compared the risk of tuberculosis in 264 patients who received HAART in phase III clinical trials and a prospective cohort of 770 non-HAART patients who were attending Somerset Hospital adult HIV clinic, University of Cape Town, between 1992 and 2001. Poisson regression models were fitted to determine risk of tuberculosis; patients were stratified by CD4 count, WHO clinical stage, and socioeconomic status.

Findings HAART was associated with a lower incidence of tuberculosis (2·4 *vs* 9·7 cases per 100 patient-years, adjusted rate ratio 0·19 [95% CI 0·09–0·38]; p<0·0001). This finding was apparent across all strata of socioeconomic status, baseline WHO stage, and CD4 count, except in patients with CD4 counts of more than 350 cells/μL. The number of tuberculosis cases averted by HAART was greatest in patients with WHO stage 3 or 4 (18·8 averted cases per 100 patient-years, adjusted rate ratio 0·22 [0·09–0·41]; p=0·03) and in those with CD4 counts of less than 200 cells/μL (14·2 averted cases per 100 patient-years, adjusted rate ratio 0·18 [0·07–0·47]; p<0·0001).

Interpretation HAART reduced the incidence of HIV-1-associated tuberculosis by more than 80% (95% CI 62–91) in an area endemic with tuberculosis and HIV-1. The protective effect of HAART was greatest in symptomatic patients and those with advanced immune suppression.

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Introduction

More than 70% of the 36·1 million HIV-1-infected individuals worldwide live in sub-Saharan Africa, and a high proportion of these are co-infected with tuberculosis.[1,2] An accelerated course of HIV-1 infection after the onset of tuberculosis has been reported in many studies.[3–6]

Tuberculosis is the leading cause of morbidity and mortality among HIV-1-infected patients in sub-Saharan Africa.[7–9] Unlike other HIV-1-related opportunistic infections, tuberculosis occurs at all levels of CD4 count,[9–11] is infectious, and its prevention is a major public-health priority.

Tuberculosis control programmes based on passive case finding and treatment of sputum-smear-positive disease by short-courses of directly observed chemotherapy (DOTS) have been successful in developed countries. However, these strategies have failed to achieve similar success in countries with high burdens of HIV-1 infection.[12,13] Consequently, WHO has formulated a strategic framework aimed at functional integration of control programmes for tuberculosis and HIV/AIDS.[14]

The survival benefits associated with highly active antiretroviral therapy (HAART) are well documented; however, studies assessing the effect of HAART on tuberculosis have shown variable results. Although some studies have shown that HAART can reduce the risk of tuberculosis by more than 80%,[15–17] others have reported no significant reduction.[18,19] No similar studies have been done in sub-Saharan Africa because only a tiny minority of the population presently has access to HAART. The UN has mobilised the Great Global Alliance to facilitate increased access to antiretroviral therapy in resource-limited settings.[20]

We did an observational study to compare the risk of tuberculosis in indigent cohorts of HIV-1-infected patients without access to HAART and in those receiving this treatment through participation in phase III randomised trials at a public health-care facility in Cape Town, South Africa.

Methods

Patients

New Somerset Hospital HIV Clinic, University of Cape Town, South Africa, is a major public health-care facility dedicated to HIV-1-infected patients in Cape Town. It was established in 1986, and serves largely indigent patients who are referred to the clinic from a wide range of primary health-care facilities in Cape Town. Antiretroviral therapy is not available in the public sector in South Africa, and patients access HAART through participation in clinical trials. Patients who expressed interest in joining the ongoing HAART clinical trials at the hospital were invited to participate on a first-come first-served basis. The desired sample

	HAART (n=264)	Non-HAART (n=770)	p
Mean (SD) age (years)	34·5 (9)	32·9 (9)	0·51
Number of women	115 (44%)	497 (65%)	<0·0001
Number with WHO stage 3 or 4	122 (46%)	227 (29%)	<0·0001
CD4 T-lymphocyte count (cells/μL)			
Median (IQR)	254 (140–364)	303 (159–468)	0·01*
<200	102 (38%)	233 (32%)	0·08
200–350	90 (35%)	189 (26%)	0·01
>350	72 (27%)	310 (42%)	<0·0001
NA	..	38 (5%)	..
Mean viral load (log_{10} [copies/μL])		5·4	NA
Number with low socio-economic status	120 (46%)	454 (59%)	0·0003

HAART=highly active antiretroviral therapy. NA=not available. *Median test.

Table 1: **Baseline demographic and clinical characteristics**

sizes were achieved in all the clinical trials. All studies were fully recruited. Participants gave informed consent, and protocols were approved by the University of Cape Town Clinical Research Ethics Committee.

Inclusion criteria common to all 12 HAART clinical trials carried out between 1995 and 2001, from which the treated cohort of this study was accrued, were: age at least 16 years; a minimum baseline plasma HIV-1 RNA concentration of 1000–5000 copies/mL (5000–30 000 copies/mL range in one study); and a CD4 count of more than 50 cells/μL (one trial), more than 100 cells/μL (three trials), more than 200 cells/μL (two trials), less than 200 cells/μL (one trial), and less than 350 cells/μL (one trial). The remaining four had no CD4 restrictions. Exclusion criteria were: acute opportunistic infection, significant laboratory abnormalities, current evidence of active substance abuse, pregnancy or lactation, and treatment with immune-modulating or systemic chemotherapeutic agents. All patients received at least three antiretroviral drugs: a non-nucleoside reverse transcriptase inhibitor with two nucleoside analogues, three nucleoside analogues, or a protease inhibitor with two nucleoside analogues. Follow-up was every 2–3 months, or more frequently if clinically indicated. At each visit, the attending health-care personnel recorded clinical, immunological, and virological information.

Patients who presented to the clinic between 1992 and 2000, and who were not participating in the clinical trials, were included in the study as a comparison group. In this cohort, patients were followed up every 3–6 months, or more frequently if clinically indicated. CD4 count was measured about every 6 months by flow cytometry. Owing to resource constraints, viral-load measurements were not available in public health-care facilities, and therefore were not analysed in this group. HIV-1 infection was confirmed by ELISA or western blot on two blood specimens. At each visit, the patient's disease was staged by use of WHO clinical criteria,[21] and laboratory data were recorded. Patients who were taking antiretroviral monotherapy or dual therapy were excluded from this study.

To account for variability in socioeconomic circumstances in the two cohorts, the Cape Metropolitan Council suburbs composite index was used.[22] This index is based on household income (proportion of households earning less than US$1500 per year), education level (proportion of adults with less than 8 years of schooling), unemployment status (unemployed adults who are actively seeking work as a proportion of all adults), welfare status (proportion of household heads who are single women with three or more children), and overcrowding status (households with more than 1·5 people per habitable room). In this composite index, a score of more than 28·5 correlated well (r=0·7) with poor living conditions,[22] and therefore patients were categorised into low or high socioeconomic status by means of this cut-off.

Further uniform exclusion criteria were applied to both cohorts. Patients were excluded if they presented with tuberculosis at their initial clinic visit, if the diagnosis of tuberculosis did not fulfil the case definition, or if they had used prophylactic isoniazid 6 months before presentation or at any time during follow-up. The tuberculosis case definition in this study was either "definite" (culture of *Mycobacterium tuberculosis* or an autopsy diagnosis of active tuberculosis) or "probable" (presence of acid-fast bacilli or a histological finding of caseating granulomata).

Statistical analysis

Differences in proportions were compared by χ^2 test, and differences in means by Student's t test. Time to tuberculosis was calculated as the time from the initial clinic visit to the date of confirmed diagnosis. Tuberculosis incidence was defined as the number of new episodes occurring in each group per 100 patient-years of follow-up. The analysis was further stratified by the baseline CD4 count, WHO clinical stage, and socioeconomic status. Number of tuberculosis cases averted by HAART was calculated with the adjusted rate ratio estimates of the Poisson multivariate regression analyses described later, and was reported with 95% CI (calculated by Poisson distribution). The choice of Poisson regression was based on the small frequency of tuberculosis events in the HAART cohort. All tests were two-sided and a p value of 0·05 was regarded as significant.

	HAART			Non-HAART			Adjusted risk ratio (95%CI)	p	Adjusted number of cases averted (95% CI)
	Number of cases of tuberculosis	Patient-years	Incidence*	Number of cases of tuberculosis	Patient-years	Incidence*			
Overall	9	375·1	2·4	82	848·2	9·7	0·19 (0·09–0·38)	<0·0001	7·3 (4·7–9·8)
CD4 count (cells/μL)									
<200	5	148	3·4	41	235	17·5	0·18 (0·07–0·47)	<0·0001	14·2 (9·7–19·7)
200–350	2	121·2	1·7	27	225	12·0	0·12 (0·03–0·53)	<0·0001	10·6 (6·8–15·9)
>350	2	100·1	2·0	14	388·3	3·6	0·36 (0·1–1·74)	0·78	2·3 (1·1–4·4)
WHO stage									
1 or 2	1	219	0·5	36	657·4	5·5	0·08 (0·01–0·57)	0·01	5·1 (3·45–7·1)
3 or 4	8	172·75	4·6	46	190·8	24·1	0·22 (0·09–0·41)	0·03	18·8 (13·2–26·1)
Socioeconomic status									
Low	6	166·21	3·6	65	514·34	10·9	0·21 (0·09–0·49)	<0·0001	8·6 (6·2–11·5)
High	3	208·89	1·44	17	333·86	5·09	0·17 (0·05–0·57)	<0·0001	4·2 (2·3–7·0)

HAART=highly active antiretroviral therapy. *Per 100 patient-years.

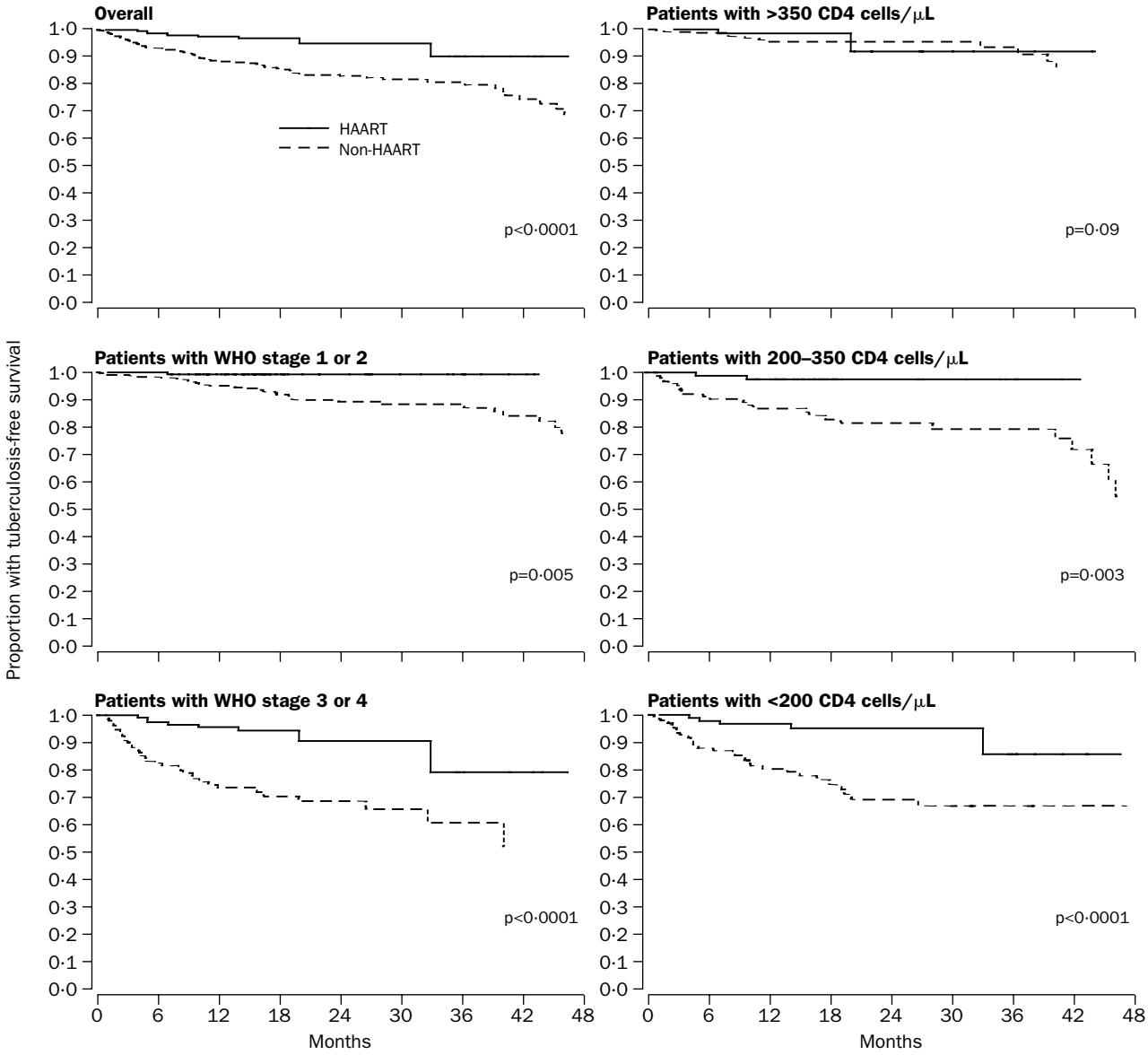
Table 2: **Tuberculosis incidence and cases averted, stratified by baseline CD4 count, WHO stage, and socioeconomic status**

The Kaplan-Meier technique and the generalised log-rank test were used to construct and compare the tuberculosis-free survival probabilities curves of the two groups. Tuberculosis-free survival was defined as the time from inclusion to the date of tuberculosis diagnosis, to death from any cause, or to the last follow-up visit. Patients who were switched over from the non-HAART to the HAART cohort contributed survival time to both cohorts: for the non-HAART cohort from their initial clinic visit to the date they started HAART, and to the HAART cohort from date of starting HAART until the date of tuberculosis diagnosis, death, or the last follow-up visit. To compare survival in the two cohorts by baseline immunological and clinical status, the Kaplan-Meier analysis was further stratified by baseline CD4 count (<200, 200–350, and >350 cells/μL) and WHO clinical stage (1 or 2, 3 or 4).

Univariate and multivariate Poisson regression models were fitted to determine risk of tuberculosis, which was expressed as a rate ratio. Sex, socioeconomic status, baseline age, year of presentation, CD4 count, and clinical WHO stage were considered for inclusion into the multivariate analysis as potential confounding variables if they were significantly associated with the risk of tuberculosis in the univariate analyses. Age was modelled as a categorical variable (less or greater than the mean age of the patient's cohort). Female sex and WHO stage 1 or 2 were modelled as baseline risk for sex and clinical WHO stage. To validate our results, we did further analyses on the subsets of patients with baseline WHO stage 1 or 2, and stage 3 or 4 separately. CD4 count was tested for normality using the Shapiro-Wilks' *W* test and was later log-transformed when found to be non-normally distributed. EpiInfo (version 6.0; CDC, Atlanta, GA, USA), STATISTICA (release 6.6, Tulsa, KA, USA), and STATA (version 6.0, College Station, TX, USA) software were used for data analysis.

Role of the funding source

The funding source had no role in the data collection, analysis, or interpretation, or the decision to submit the study for publication.



Kaplan-Meier probabilities of tuberculosis-free survival

	Rate ratio (95% CI)			
	Univariate analysis	p	Multivariate analysis	p
General cohort				
HAART	0·25 (0·13–0·50)	0·0001	0·19 (0·09–0·38)	<0·0001
CD4 count (log_{10} baseline)	0·45 (0·11–0·62)	<0·0001	0·67 (0·47–0·99)	0·03
WHO stage (3 or 4)	3·48 (1·17–5·29)	<0·0001	4·28 (2·64–6·95)	<0·0001
Low socioeconomic status	1·67 (1·02–2·59)	0·02	1·59 (1·01–2·50)	<0·0001
WHO stage 1 and 2				
HAART	0·10 (0·01–0·74)	0·02	0·07 (0·009–0·55)	0·01
CD4 count (log_{10} baseline)	0·28 (0·13–0·59)	0·001	0·18 (0·08–0·43)	<0·0001
Low socioeconomic status	1·56 (0·77–3·16)	0·22	1·39 (0·69–2·84)	0·35
WHO stage 3 and 4				
HAART	0·19 (0·09–0·42)	<0·0001	0·21 (0·10–0·46)	0·039
CD4 count (log_{10} baseline)	0·89 (0·56–1·45)	0·66	0·89 (0·57–1·42)	0·65
Low socioeconomic status	2·17 (1·23–3·81)	0·008	1·83 (1·03–3·24)	0·03

HAART=highly active antiretroviral therapy.

Table 3: **Poisson regression analyses for predictors of tuberculosis**

Results

1085 patients in the non-HAART cohort and 270 patients in the HAART cohort were studied. 315 patients were excluded from the non-HAART cohort: 79 were on antiretroviral monotherapy or dual therapy, isoniazid prophylaxis, or both; 222 presented with tuberculosis at their initial clinic visit; and 14 incident cases received tuberculosis chemotherapy but did not meet the tuberculosis case definition. The remaining 770 patients were included in the analysis. Of the 270 patients recruited in the HAART trials, two patients who presented with tuberculosis at their initial clinic visit, and four who started tuberculosis chemotherapy but did not meet tuberculosis case definition were excluded from the study. The remaining 264 patients included in the analysis received HAART. 40 patients who started off in the non-HAART cohort switched to the HAART cohort.

The baseline demographic and clinical characteristics of both cohorts are shown in table 1. Mean age in the two groups did not differ significantly, but the proportion of women in the non-HAART cohort was significantly higher than in HAART cohort, probably due to the systematic exclusion of pregnant or lactating women in the HAART cohort. At baseline, the HAART cohort had more clinical advanced HIV-1 disease and lower CD4 counts than the non-HAART cohort. Baseline CD4 count was not available for 38 patients in the non-HAART cohort.

Mean follow-up in the HAART cohort was significantly greater than in the non-HAART cohort (16·8 months [SD 8·3] *vs* 13·2 months [15·5]). During follow-up, nine cases of tuberculosis (four probable and five definite) were reported in the HAART cohort compared with 82 cases (48 probable and 34 definite) in the non-HAART cohort (unadjusted rate ratio 0·15 [95% CI 0·08–0·32]; p<0·0001, table 2). The rate ratio remained significant when patients were stratified by baseline WHO stage or CD4 count, except in the stratum of patients with CD4 count of more than 350 cells/μL. The greatest number of tuberculosis cases averted by HAART was in the subset of patients with baseline WHO stage 3 or 4 (table 2).

A similar trend to that reported in the above stratified incidence analysis was seen in the tuberculosis-free survival proportions in the stratified Kaplan-Meier analysis of the two cohorts shown in the figure. Overall median tuberculosis-free survival in the HAART cohort was significantly greater than that of the non-HAART cohort, and across all strata of WHO stages and CD4 counts, but not in the stratum of more than 350 CD4 cells/μL.

We did a separate analysis to ascertain the outcome of the 38 patients with missing baseline CD4 count in the non-HAART group. The proportion of tuberculosis cases occurring in this group (five of 38 [13%]) was not significantly different from that of patients in the non-HAART cohort for whom baseline values were available (82 of 770 [11%]; p=0·8). Tuberculosis-free survival was also similar in the two groups (p=0·42).

Poisson multivariate regression analysis revealed that, after controlling simultaneously for baseline differences, HAART conferred an independent protective benefit against risk of tuberculosis (table 3). Other predictors of tuberculosis were WHO stage 3 or 4, low socioeconomic status, and baseline CD4 count (table 3). In the univariate analyses, sex (female, rate ratio 1·45 [95% CI 0·93–2·28]; p=0·07) and age (greater or less than mean age, 0·78 [0·51–1·20]; p=0·09), and year of presentation (0·99 [0·91–1·08]; p=0·87) were not significantly associated with the risk of tuberculosis and were thus not included in the multivariate analysis.

In a separate subset, an independent and consistent protective benefit of HAART was seen in multivariate analyses of patients with baseline WHO stage 1 or 2 or stage 3 or 4 (table 3). The adjusted risk of tuberculosis associated with CD4 count (log_{10} baseline) was significant in the multivariate analysis of patients with baseline WHO stage 1 or 2, but not in patients with baseline WHO stage 3 or 4. Conversely, low socioeconomic status was associated with increased risk of tuberculosis in the subset analysis of WHO stage 3 or 4 but not of WHO stage 1 or 2 (table 3).

Discussion

We have shown a substantial reduction in tuberculosis incidence attributable to HAART in HIV-1-infected individuals in sub-Saharan Africa. This study differs from previous reports because the high frequency of tuberculosis in our cohort allowed quantification of the protective effect of HAART at the different stages of HIV-1 disease. The effect of HAART was significant across all the baseline immunological, clinical, and socioeconomic variables in our cohort, except in patients with CD4 counts of more than 350 cells/μL. The greatest number of tuberculosis cases averted by HAART was in patients with baseline WHO clinical stage 3 or 4 and those with CD4 counts of less than 200 cells/μL.

The overall tuberculosis risk reduction estimate of 81% (95% CI 62–91) associated with use of HAART in this study is similar to that reported in two studies from the USA and Italy (80% and 92%, respectively).[16,17] Brodt and colleagues[18] found no significant tuberculosis

reduction during a 5-year period in which HAART use in a German cohort increased from 5·7% to 87·3%. Moreover, although there was a tendency towards a lower risk of tuberculosis with HAART use in a study by Santoro-Lopes and colleagues from Brazil,[19] the adjusted estimate was not significant. However, only 41 individuals received HAART in that study.

The number of tuberculosis cases averted is a function of both risk reduction due to HAART and tuberculosis incidence. The overall tuberculosis incidences in the studies from the USA and Italy were low (0·5 and 0·79 per 100 patient-years, respectively);[16,17] rates were higher in the studies by Brodt and colleagues (2·1 per 100 patient-years)[18] and Santoro-Lopes and colleagues (8·4 per 100 patient-years)[19] because only patients with advanced HIV-1 disease (CD4 counts <200 cells/μL and <15%, respectively) were enrolled. Although the overall tuberculosis incidence in our study was 7·4 per 100 patient-years, patients with CD4 counts of less than 200 cells/μL had a tuberculosis incidence of 12 per 100 patient-years, which is a substantially higher rate than those reported in these studies.

Owing to current international funding initiatives and decreasing cost, access to HAART is expected to increase in resource-constrained countries. Our results are of particular relevance to the recently proposed WHO guidelines for the scaling up of antiretroviral therapy in resource-limited settings. These guidelines recommend starting antiretroviral therapy in adult and adolescent HIV-1-infected patients with WHO stage 4 disease or a CD4 count of 200 cells or fewer per μL.[20] In the light of these recommendations, our analyses suggest that starting HAART in these two groups will result in prevention of 14–20 cases of tuberculosis per 100 patient-years of treatment. However, tuberculosis incidence remained high in these groups and in individuals with CD4 counts of between 200 and 350 cells/μL, despite the substantial numbers of tuberculosis cases averted by HAART.

Initiation of HAART in patients with CD4 counts of less than 350 cells/μL will have large cost implications, particularly in resource-constrained settings. Therefore, tuberculosis preventive therapy, which has been reported to reduce the risk of tuberculosis in HIV-1-infected patients with a tuberculin-positive skin test (PPD) by more than 40%,[23,24] might be a more attractive alternative for reducing risk of tuberculosis in patients with CD4 counts between 200 and 350 cells/μL.

In multivariate analysis, low socioeconomic status was independently associated with increased risk of tuberculosis in the general cohort and in patients with baseline WHO stage 3 or 4, but not in those with baseline WHO stage 1 or 2. 56% of our cohort were socially deprived and lived in areas characterised by high rates of tuberculosis infection and poor living conditions. These factors, compounded with advanced HIV-1 disease, resulted in extremely high tuberculosis rates.

Our study has some limitations. Tuberculosis prophylaxis of PPD-positive individuals was not part of the national tuberculosis control programme during the study period, and PPD test results were not available in our cohort. Latent tuberculosis infection is, however, prevalent in our local population. In more than 900 antiretroviral-therapy-naive patients with confirmed HIV-1 infection at a voluntary counselling centre in Cape Town, PPD positivity was 55%.[25] We have adjusted in our multivariate analysis for factors known to be associated with overt tuberculosis disease in our setting.[26]

The observational design of our study is a further limitation, but because of the recognised survival benefits of HAART, a randomised placebo-controlled trial in patients with advanced HIV-1-disease at high risk of tuberculosis would not be ethically justifiable. The two cohorts in our study were largely self-selected and the HAART cohort was under trial-determined conditions with fairly intense follow-up that might lead to greater opportunity to diagnose tuberculosis. Unmeasured factors such as viral load, which was not available for the non-HAART cohort, could account for some of the higher tuberculosis incidence in this group. The two cohorts were not strictly contemporaneous, but year of presentation was not a significant factor for the risk of tuberculosis in our analysis. Data on baseline CD4 count were not available for 38 patients in the non-HAART cohort. However, survival and tuberculosis incidence in this group was not different from that of patients in whom baseline CD4 counts were available. The median follow-up in our cohort was limited; however, the number of tuberculosis events was higher than that reported in all previous studies.

In conclusion, our study has quantified the added benefit of tuberculosis reduction that would result from expanded access to HAART, as proposed by WHO, in a setting of high tuberculosis and HIV-1 prevalence. The decrease in tuberculosis incidence with HAART was substantial, but immune-compromised and symptomatic individuals were still at unacceptably high risk of developing active tuberculosis. Tuberculosis preventive therapy remains an important strategy for patients with early HIV-1 disease. However, because social deprivation was shown to be a significant risk factor for tuberculosis in this group, medical interventions cannot be separated from the need for social improvement. Our findings suggest that HIV-1 control is required for effective tuberculosis control, and that HAART can have a critical role in addressing the therapeutic nihilism surrounding the HIV-1 and tuberculosis co-epidemic in South Africa and other African countries.

Contributors

All authors contributed to conceptualisation, design, data collection, and revision of the final draft of the study, which was written by M Badri. R Wood contributed to the design of the statistical analysis. M Badri designed and carried out the statistical analysis.

Conflict of interest statement

None declared.

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QAD 0354

EDITORIAL REVIEW

How effectively does HAART restore immune responses to *Mycobacterium tuberculosis*? Implications for tuberculosis control

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Use of highly active antiretroviral treatment (HAART) has had a major impact on HIV-associated morbidity and mortality in industrialized countries. Access to HAART is now expanding in low-income countries where tuberculosis (TB) is the most important opportunistic disease. The incidence of TB has been fuelled by the HIV epidemic and in many countries with high HIV prevalence current TB control measures are failing. HAART reduces the incidence of TB in treated cohorts by approximately 80% and therefore potentially has an important role in TB control in such countries. However, despite the huge beneficial effect of HAART, rates of TB among treated patients nevertheless remain persistently higher than among HIV-negative individuals. This observation raises the important question as to whether immune responses to *Mycobacterium tuberculosis* (MTB) are completely or only partially restored during HAART. Current data suggest that full restoration of circulating CD4 cell numbers occurs only among a minority of patients and that, even among these, phenotypic abnormalities and functional defects in lymphocyte subsets often persist. Suboptimal restoration of MTB-specific immune responses may greatly reduce the extent to which HAART is able to contribute to TB control at the community level because patients receiving HAART live much longer and yet would maintain a chronically heightened risk of TB.

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Keywords: HIV, *Mycobacterium tuberculosis*, tuberculosis, antiretroviral, HAART, immune reconstitution

Introduction

Since the mid 1990s, use of highly active antiretroviral treatment (HAART) has been associated with a dramatic decline in HIV-associated morbidity and mortality in many high-income countries [1–3]. Suppression of viral replication permits both quantitative and functional reconstitution of the immune system [4–6]. Primary and secondary prophylaxis for opportunistic pathogens such as cytomegalovirus, *Pneumocystis jirovecii*, *Mycobacterium avium complex* (MAC), *Toxoplasma gondii* and *Cryptococcus neoformans* can be discontinued as functional host responses to these organisms are gradually restored [7–10]. The risk of recurrence of these opportunistic

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infections is generally very low once blood CD4 cell counts have reached stable levels $> 200 \times 10^6$ cells/l [7]. Thus, substantial clinical benefit is achieved without full restoration of circulating CD4 cell numbers. It remains unclear, however, whether long-term viral suppression by HAART can effect complete quantitative and functional restoration of the immune system. Moreover, the optimal timing for initiation of HAART to preserve capacity for restoration remains to be determined.

The extent of immune restoration has important implications for the pattern of opportunistic infections occurring during HAART. Partial immune restoration may be sufficient to prevent disease from low virulence opportunistic pathogens such as disseminated MAC that develops exclusively in patients with profound immunodeficiency. However, more virulent pathogens such as *M. tuberculosis* (MTB) cause disease across the full spectrum of HIV-associated immunodeficiency [11] and restoration of MTB-specific functional immune responses that is only partial will result in a persistently heightened risk of tuberculosis (TB). The differential impact of HAART on host responses to pathogens has contributed to major changes in disease epidemiology in the HAART era. For example, TB is now the most frequent HIV-associated mycobacteriosis in Europe whereas the incidence of disseminated MAC was twice that of TB in the pre-HAART era [12].

Access to HAART is expanding in many low-income countries where the spectrum of opportunistic infections differs from that in high-income countries [13]. It is not yet clear how HAART will impact this burden of HIV-associated disease. TB is the opportunistic infection of prime importance and is the leading cause of morbidity and mortality among HIV-infected patients living in sub-Saharan Africa [14,15]. An estimated 31% of cases and 39% of TB deaths are attributable to HIV [16] and case series indicate that up to 80% of patients with TB are co-infected with HIV. In some townships in Cape Town, South Africa, HIV prevalence rates among antenatal women are approximately 30% and the incidence of TB in these communities has increased to around 1400/100 000 per year (unpublished data). Among HIV-infected patients with World Health Organization (WHO) stage 3 and 4 disease in Cape Town, the incidence of TB is extremely high with 24.1 cases per 100 patient-years [17].

The existing TB control strategy of case-finding and directly observed treatment of sputum smear-positive patients using short-course antituberculosis treatment is not proving adequate in countries with a high burden of HIV [18,19]. WHO has therefore formulated a strategic framework aimed at functional integration of control programmes for TB and HIV/AIDS [20]. HAART is one element within this framework. However, whether widespread use of HAART will prove an effective tool in TB control at the community level is not yet known.

The extent to which HAART will have an effect on TB control will be determined, in part, by the rate and extent to which functional immune responses to MTB are restored. Epidemiological, clinical and laboratory data all provide evidence that HAART restores host responses to MTB. These data include: (i) reductions in incidence of TB; (ii) alterations in the clinicopathological features of the disease; (iii) changes in skin test responses to purified protein derivative (PPD); and (iv) laboratory data showing improvements in functional responses of circulating lymphocytes. However, data are also emerging that suggest that immune restoration during HAART is incomplete and that an ongoing heightened risk of TB persists during long-term treatment. The magnitude of this risk is likely to reflect time-dependent changes in host immune function. Here we review these data and discuss the potential of HAART in TB control.

Impact of HAART on the incidence of TB

With one exception [21], studies conducted in countries with low [12,22–24] or high [17,25] prevalence of TB have shown that HAART is associated with substantial reductions in risk of TB in the order of 70–90% among HIV-infected cohorts (Table 1). In Cape Town, South Africa, this impact was significant across all the baseline immunological, clinical and socioeconomic variables, except for patients with blood CD4 cell counts $>350 \times 10^6$ cells/l [17]. The greatest number of TB cases averted by HAART was in patients with baseline WHO clinical stage 3 and 4 and those with CD4 cell counts of $<200 \times 10^6$ cells/l (Fig. 1). These studies suggest that expanding access to HAART may have a beneficial impact on TB control in high prevalence countries.

However, emerging data also suggest that although TB rates in treated cohorts decrease following initiation of HAART, rates remain persistently higher than among HIV-negative individuals. This observation has been made in countries with a low and high burden of TB. Among the Swiss cohort, in which the median pre-HAART CD4 cell count was 188×10^6 cells/l, an elevated though diminishing rate of TB was observed for at least the first 6 months of HAART [22]. Certainly a lag time exists between the initiation of HAART and the restoration of sufficient levels of anti-mycobacterial immune function that are able to exert a protective effect. High but diminishing rates of TB after commencement of HAART are likely to reflect time-dependent reductions in residual immunodeficiency, the duration and extent of which may well depend on the nadir CD4 cell count. Immune reconstitution disease (IRD) may also

Table 1. The impact of HAART on the incidence of tuberculosis (TB) in cohort studies.

Study	Country	Study population	Outcome
Studies in countries with high TB incidence			
Badri *et al*. 2002 [17]	South Africa	Prospective case-control cohort study (264 receiving HAART, 700 non-HAART), 1992–2001	Adjusted rate ratio for TB was 0.19 (95% CI, 0.09–0.38) among those receiving HAART
Santoro-Lopes *et al*. 2002 [25]	Brazil	Prospective cohort (n = 255), 1991–1998	Relative hazard of TB among those receiving triple-therapy was 0.19 (95% CI, 0.03–1.09) compared to those receiving monotherapy or no treatment
Studies in countries with low TB incidence			
Brodt *et al*. 1997 [21]	Germany	Prospective cohort of homosexual men (n = 1003), 1992–1996	No change in TB incidence during HAART despite major reductions in other opportunistic infections
Girardi *et al*. 2000 [23]	Italy	Multicentre prospective cohort (n = 1360), 1995–1996	Relative hazard of TB among those receiving HAART was 0.08 (95% CI, 0.01–0.88), compared to those not receiving HAART
Jones *et al*. 2000 [24]	USA	Multicentre prospective cohort study 1996–1998	Relative risk of TB among persons receiving HAART was 0.2 (95% CI, 0.1–0.5) compared to those not receiving HAART
Kirk *et al*. 2000 [12]	17 European countries	EuroSIDA multicentre prospective cohort (n > 7000)	TB incidence decreased from 1.8 cases/100 py to 0.3 cases/100 py during HAART.

HAART, Highly active antiretroviral therapy; CI, confidence interval; py, person years.

contribute to TB incidence in the initial months of HAART (see below); active but subclinical TB among profoundly immunodeficient patients may manifest as host immune responses are restored [26,27].

An increased rate of TB during HAART is not simply a short-term phenomenon. An observational study in Italy of HIV-associated TB in the era of HAART described factors associated with 271 cases incident cases [28]. Of these, 30.3% were receiving HAART prior to TB diagnosis and this treatment had been prescribed for a median of 27 months. The median blood CD4 cell count at diagnosis of TB (220×10^6 cells/l) was significantly greater than the level pre-HAART (80×10^6 cells/l) and was also significantly greater than the median CD4 cell count of HIV-infected patients who developed TB but were not receiving HAART (109×10^6 cells/l). This finding suggests that substantial restoration of CD4 cell numbers had occurred among the majority of patients receiving HAART by the time TB was diagnosed.

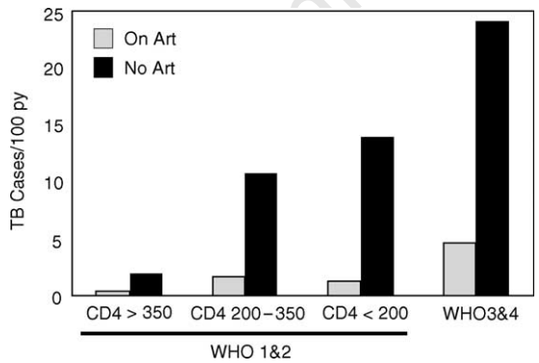

Fig. 1. The incidence of TB [cases per 100 person-years (py)] among cohorts of HIV-infected patients in Cape Town, South Africa who were or were not receiving antiretroviral treatment (ART). Data are stratified by blood CD4 cell count ($\times 10^6$ cells/l) and WHO clinical stage of disease (Adapted from Badri *et al*. 2002 [17]).

Similar observations are also being reported from the developing world. TB was the most common opportunistic infection in a cohort of patients with advanced immunosuppression receiving HAART in Thailand [29]. Among patients with WHO stage 3 and 4 disease in Cape Town, South Africa, the incidence of TB during HAART was 4.6 cases/100 patient-years; this is approximately 10-fold greater than the incidence among HIV-negative individuals in the same community [17]. A pilot community-based antiretroviral project in Cape Town reported high rates of TB during the first year of HAART [30]. Our current observations at the same site are that among patients referred for HAART, approximately 5% have active TB on pre-HAART screening and a further 10% of patients develop TB after initiation of HAART during the first year of follow up (unpublished data) despite excellent treatment compliance [31]. Thus, although TB rates are markedly reduced by HAART, TB still represents a substantial burden of disease during this treatment, especially in communities with high TB prevalence.

Impact of HAART on the manifestations of TB

Clinical and radiological presentation

The impact of HIV on the clinicopathological features of TB is well documented [32,33]. HIV-associated

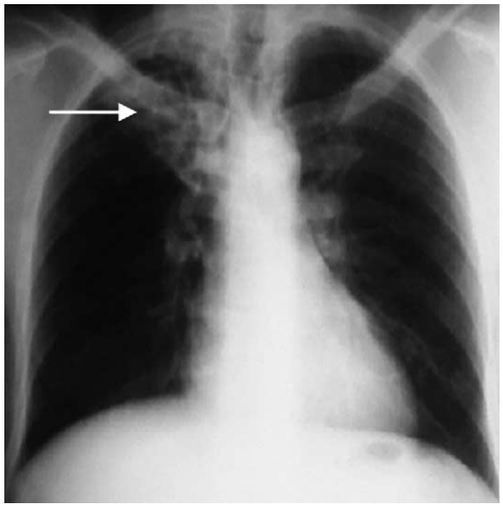

Fig. 2. Chest radiograph of an HIV-infected patient with pulmonary TB during HAART. Despite previously advanced immunodeficiency, restoration of immune function during HAART may lead to an increased frequency of typical post-primary apical cavitating disease as shown in this patient at the right lung apex (arrow).

immunodeficiency results in an increased frequency of cutaneous anergy to PPD and increased rates of extrapulmonary and disseminated disease [32,34]. Radiographic appearances of pulmonary TB in patients with HIV-1 coinfection are more frequently atypical, reflecting impaired tissue inflammatory responses to infection [35,36]. All-cause mortality is also greatly increased and is directly related to the degree of immunodeficiency [37]. However, as might be expected, HAART modifies the clinicopathological features of TB as immune function is restored. HAART increases the frequency of the typical post-primary radiographic pattern of pulmonary TB (Fig. 2) [38,39], rates of PPD skin test conversion (Fig. 3) are higher [40,41] and patient survival is increased [38]. The frequency of extrapulmonary disease might also be expected to decrease among patients receiving HAART, but the single study examining this possibility was not of sufficient size for full evaluation [38].

Immune reconstitution disease

The most striking evidence of restoration of functional immune responses to MTB is that provided by reports of IRD, also known as the immune reconstitution inflammatory syndrome. IRD is an adverse consequence of restoration of immune responses during the initial months of HAART [26,27]. Previously subclinical infections are 'unmasked' or pre-existing partially treated opportunistic infections clinically deteriorate as immunopathological host inflammatory responses are 'switched on' during HAART. IRD associated with HAART was originally described in patients with MAC infection and who were receiving zidovudine monotherapy [41].

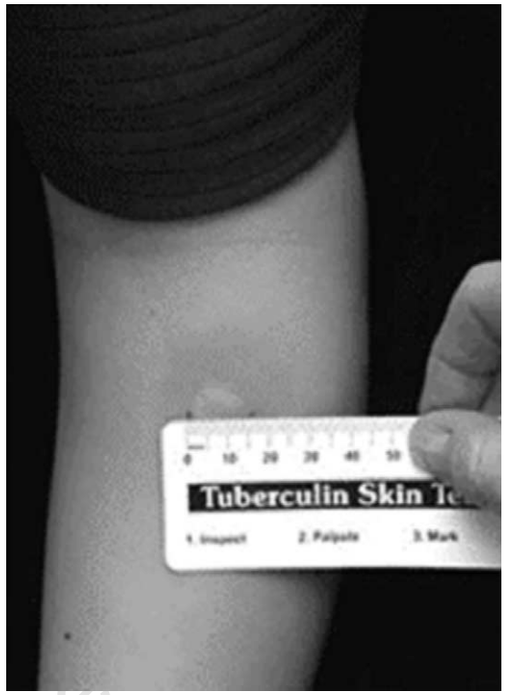

Fig. 3. Conversion of a DTH skin test response to tuberculin from anergic to positive may reflect restoration of cell-mediated immune responses to *M. tuberculosis* and may also accompany immune reconstitution disease associated with this organism. (Photograph courtesy of Division of Tuberculosis Elimination, National Center for HIV, STD and TB Elimination, Centers for Disease Control and Prevention, Atlanta, GA, USA).

Presentation with IRD was temporally associated with development of positive PPD skin test responses (Fig. 3) among patients with previous cutaneous anergy, providing evidence of restoration of delayed type hypersensitivity (DTH) responses.

IRD associated with MTB was not reported until after the development of HAART [42]. Among 86 published cases of TB-associated IRD, the median duration of HAART prior to development of IRD was just 4 weeks, illustrating the extraordinary rapidity with which HAART reverses HIV-associated paresis of immune responses to MTB [26]. Among these cases, the median blood CD4 cell count increased from a pre-treatment nadir of 5×10^6 cells/l [interquartile range (IQR), $26\ 10^6 - 103 \times 10^6$ cells/l] to 205×10^6 cells/l (IQR, $110\ 10^6 - 298 \times 10^6$ cells/l) at the time of IRD diagnosis. Typically the plasma viral load is markedly reduced by the time of IRD onset, reaching the lower limit of detection in approximately half the patients. Some patients develop IRD associated with TB within 2 weeks of initiating HAART, even prior to an increase in CD4 cell count. This finding is likely to reflect the rapid restoration of functional immune responses that accompanies rapid reductions in viral load.

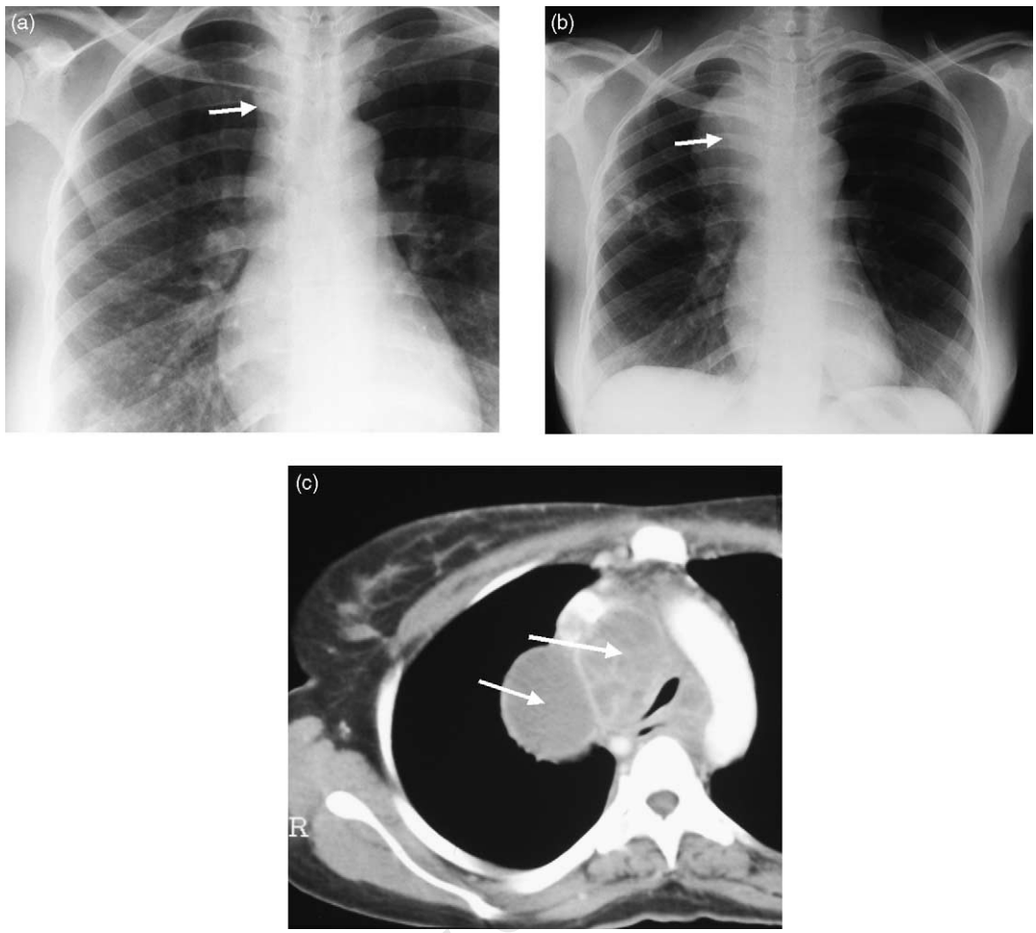

Fig. 4. Immune reconstitution disease associated with *M. tuberculosis*. (a) Chest radiograph of an HIV-infected patient showing right paratracheal lymphadenopathy (arrow) and a reticulonodular infiltrate due to *M. tuberculosis* infection. (b) After 2 months of anti-TB therapy and HAART, the patient presented with stridor. A repeat chest radiograph shows massive right paratracheal adenopathy (arrow) displacing and compressing the trachea, minor right hilar adenopathy and a nodular infiltrate in the right mid-zone. (c) Computed tomography shows enlarged lymph nodes in the right paratracheal region (arrows) causing displacement and marked compression of the trachea. (Reproduced with permission from [51]).

MTB-associated IRD most commonly presents with fever, lymphadenopathy or worsening respiratory symptoms. A wide spectrum of other manifestations include development of pleural effusions, ascites, hepatosplenomegaly [43], psoas abscesses [44], intra-abdominal abscesses [45], epididymo-orchitis [43], central nervous system lesions [46], skin lesions [42], acute renal failure [47] and hypercalcaemia [48]. These observations indicate that HAART rapidly restores anti-mycobacterial immune function in diverse anatomical compartments and suggests that HAART permits recruitment and proliferation of effector cells at those sites. Rapid reversal of immune function may trigger severe immunopathology in patients with subclinical or partially treated TB. Life-threatening clinical manifestations include acute respiratory failure from widespread pulmonary alveolitis [49,50] and major airway obstruction due to rapidly expanding intra-thoracic lymph nodes (Fig. 4) [51]. Systemic corticosteroid treatment plays a key role in the control of severe manifestations.

Histopathological examination of tissues involved in IRD associated with mycobacteria may show suppuration and a degree of necrotizing inflammation that is unusual in the context of profoundly immunocompromised patients [52–54]. It is likely that the rapidity with which immune responses are restored and the lack of compensating immunoregulatory mechanisms result in the uncontrolled tissue-damaging responses that characterize IRD. Further indirect evidence that HAART restores the ability to form physiologically functioning granulomas (Fig. 5) is provided by reports of hypercalcaemia during mycobacterial IRD [48,55]. Hypercalcaemia is a well-recognized complication of mycobacterial infection, arising as a result of production of 1,25-dihydroxycholecalciferol in functional granulomas [56]. Elevated serum levels of

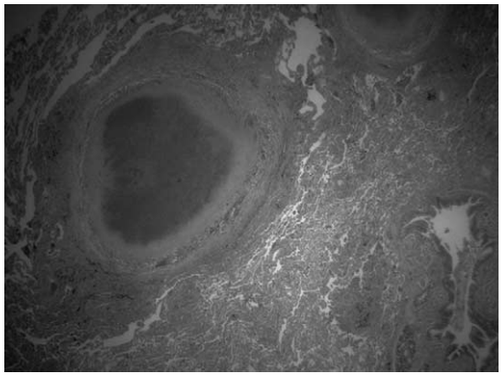

Fig. 5. Haematoxylin and eosin stained tissue section showing a florid granulomatous host response to *M. tuberculosis*. As immune responses recover during HAART, the ability to form granulomas is restored. (Photomicrograph courtesy of the Division of Anatomical Pathology, Faculty of Health Sciences, University of Cape Town, South Africa).

1,25-dihydroxycholecalciferol and hypercalcaemia during mycobacterial IRD is therefore likely to reflect restoration of the ability to form physiologically functioning granulomas [48].

Mechanisms and extent of immune recovery during HAART

The development of HAART using combinations of reverse transcriptase and protease inhibitors in 1995 marked the dawn of a new era in HIV management. For the first time, robust suppression of plasma viral load was associated with substantial immune restoration [4–6]. HAART usually leads to a >90% reduction in plasma viral load within the first weeks of treatment [57]. Three principal laboratory parameters provide evidence of subsequent immune recovery during HAART: (i) quantitative restoration of immune cells; (ii) normalization of cell phenotype; and (iii) recovery of immune cell function. Here, we initially consider immune recovery in general and later review the data that specifically relate to immunity to MTB.

Changes in CD4 cell counts

The CD4 cell count increases that accompany the viral load reductions occur in two principal stages. A rapid increase in the number of circulating CD4 cell lymphocytes within 1–2 weeks of starting HAART and continuing over the first 2–3 months largely represents a redistribution of activated CD45Ro memory cells previously sequestered in lymphoid tissue, and a generalized reduction in apoptotic cell death [4,5]. This initial increase coincides with the period within which manifestations of IRD are most frequently observed. A slower second phase of CD4 cell expansion persists over subsequent months and may continue for years. This observation represents expansion of naive CD45RA cells as thymic function is restored and results in the long-term sustained rise in CD4 cell count. This sustained increase in CD45RA cells correlates with the magnitude of viral load suppression and its stability over time [5].

After 2–4 years of HAART, mean or median increases in CD4 cell counts are approximately $200–400 \times 10^6$ cells/l [58–62]. The increases predominantly occur in the first 1 or 2 years with comparatively smaller gains thereafter. In a multicentre study of 314 HIV-infected homosexual men in the USA, blood CD4 cell counts were observed to plateau between 2 and 3.5 years of HAART [63]. In a prospective cohort of 237 HAART-naive patients, with a baseline median CD4 cell count of 175×10^6 cells/l and viral suppression maintained for more than 1 year, more sustained CD4 lymphocyte increases were observed during HAART [61]. After an initial rapid increase in median CD4 cell count of 97.2×10^6 cells/l in the first month, the rate of CD4 cell increase sequentially diminished thereafter, with rates of 175×10^6 cells/l/month from year 1 to year 2 and an estimated rate of 5.4×10^6 cells/l/month at year 2. A more recently published study with 6 years follow-up of 20 selected patients found that CD4 cell counts continued to rise, albeit slowly, during years 3–6 of HAART [64].

While viral suppression is a key determinant of long-term CD4 cell recovery [62], the nadir CD4 cell count at the time HAART is started may also be an important factor. CD4 cell repletion in both blood and lymphoid compartments occurred during the first year of HAART among patients with early disease and baseline CD4 cell counts $> 400 \times 10^6$ cells/l [65]. In contrast, CD4 cell numbers had not normalized after nearly 3 years of follow up among patients with severely depleted CD4 cell counts at initiation of treatment [66]. A more recently published study of a small group of patients, however, showed that CD4 cell rises may continue for up to 6 years despite severe pre-treatment CD4 cell depletion [64]. Kaufmann *et al*. found that older age and lower nadir CD4 cell counts were both predictive of a poorer CD4 cell recovery [59] and these factors may be related to persistent impairment of thymic function [67]. The extent of CD4 cell recovery may also be related to low-level viraemia and pro-viral DNA levels [68]. It can be concluded that responses to HAART fall within a spectrum. Some patients have little or no rise in CD4 cell counts despite complete viral suppression [69,70]; others may have CD4 cell counts that plateau at suboptimal levels [63] and a proportion have persistent CD4 cell increases over many years [64].

Changes in CD4 cell phenotype

The phenotype of circulating T lymphocytes in HIV-infected patients reflects immune competence. Numbers

of studies show that circulating CD4 and CD8 lymphocyte subsets are only partially restored in the first year of HAART [4,71,72]; as with total CD4 cell counts, however, changes in these subsets may continue for many years [64]. Although expression of the cellular activation markers, HLA-DR and CD38, by CD4 and CD8 lymphocytes decreases substantially during the first year of HAART, these populations remain abnormally activated for up to 6 years of HAART [60,64]. Furthermore, the number of circulating CD4 and CD8 lymphocytes that express CD28, a co-stimulatory molecule important in T-cell responses to antigen, do not increase to normal levels during long-term HAART even among those who normalize their CD4 cell counts [73]. Additionally, HLA-DR and CD38 expression by CD8 lymphocytes remain elevated among patients starting HAART with advanced disease. Overall, among patients with virological suppression, the likelihood of phenotypic normalization of circulating lymphocytes diminishes the lower the nadir CD4 cell count [73].

Changes in CD4 cell function

In addition to demonstrating quantitative cellular restoration and improvements in immune cell phenotype, a number of studies has also described improvements in immune function during HAART [4,60,72–74]. In general, *in vitro* T-cell proliferative responses to recall antigens and mitogens improve during treatment [4,60,72] and cytokine responses shift from a predominantly type 2 to a type 1 pattern, with increases in interferon (IFN)-γ and interleukin (IL)-2 production [75–77]. Later increases in circulating CD45RA naive lymphocytes are associated with reversal of HIV-associated defects in the T-cell receptor (TCR) repertoire [78,79] and restoration of immune responses to neoantigens [74]. DTH responses to antigens assessed by skin testing also improve and correlate with the magnitude of viral load reduction [80].

It is clear, however, that long-term functional deficits persist during HAART. Imbalances in cytokine profile and TCR repertoire disruptions persist for at least 1 year of treatment [77,79] and antibody responses to vaccines, such as pneumococcal vaccine, remain suboptimal during long-term treatment [81]. Lange *et al.* conducted a detailed assessment of functional T-cell responses among patients who had a wide spectrum of nadir blood CD4 cell counts, good virological responses and normalized CD4 cell counts on long-term HAART [82]. Antibody responses to immunizations, lymphocyte proliferation *in vitro* and DTH responses to vaccine antigens were evaluated. Despite normal CD4 cell counts, functional immune responses remained impaired and the degree of impairment was directly related to the nadir but not current CD4 cell count [73].

Restoration of MTB-specific immune responses during HAART

Few studies examine restoration of anti-mycobacterial and MTB-specific immune responses. Among a cohort of patients with a history of disseminated MAC infection, proliferation of peripheral blood mononuclear cells (PBMC) in response to MAC antigen *in vitro* showed stimulation indices of ≥ 3 among 46% of patients prior to HAART and among 77% of patients after 6 months of HAART [83]. There was no further increase in the proportion of patients with positive responses at 12 months and the proportion of positive responders was similar to that among HIV-negative control subjects. Similar data are reported by Wendland *et al.* [80]. Restoration of proliferative responses to MAC and *M. xenopi* during the first 6 months of HAART is temporally associated with development of IRD [84].

Proliferation of PBMC stimulated *in vitro* with PPD is also enhanced during receipt of HAART; the proportion of positive responders increases steeply during the first 6 months [4,72,80] and is sustained at 12 months [72]. Li *et al.* showed that not just the proportion of responders but also the magnitude of cell proliferation increases markedly [72]. In a prospective cohort of 10 patients followed up at 2-monthly intervals over 1 year, Schluger *et al.* found that PBMC stimulated with the laboratory strain MTB H37Ra also produced a steadily increasing amount of IFN-γ [85]. However, responses at 1 year were markedly less than those among HIV-negative control groups with either positive or negative PPD skin test responses. Furthermore, production of IL-2 and IL-12 increased minimally.

Hsieh *et al.* prospectively studied 13 patients during HAART for 1 year, using expression of the lymphocyte activation marker CD69 following *in vitro* stimulation of PBMC with PPD as a correlate of MTB-specific immune responses [86]. In a majority of patients, the percentage of CD4 cells and CD8 cells responding to PPD increased during the first year of HAART. However, although substantial increases in CD4 cell count were observed in all patients, a small group of patients failed to restore these responses after 1 year of HAART. These patients were more likely to have nadir CD4 cell counts of $<50 \times 10^6$ cells/l compared to those who had good restoration of responses [86]. This suggests that delaying initiation of HAART in chronic HIV infection may lead to long-term impairment of functional immune responses to MTB.

Quantitative restoration of MTB-specific IFN-γ-secreting cells during HAART has not been investigated. Hengel *et al.* described a single patient with disseminated MTB infection and HIV infection who started anti-tuberculosis treatment and HAART concurrently [87]. The proportion of peripheral blood MTB-specific CD4

cells staining positively for IFN-γ increased from 8.6% on day 11 to 33% on day 95 of treatment. To what extent this remarkable expansion of MTB-specific T cells represented an effect of HAART or an effect of anti-tuberculosis treatment is unknown. Studies are needed to determine the extent to which MTB-specific T cell clones are restored during HAART.

HAART as a TB control strategy

Although finding and curing active TB cases remains the principal intervention for TB control in high HIV prevalence settings [88] it is nevertheless failing [18,19]. A multifaceted approach is therefore required [20]. The extent to which HAART will play a role within such a framework has yet to be determined and will depend on: (i) the efficacy of HAART in preventing TB; (ii) the population coverage; and (iii) the extent to which HAART prolongs life. It seems likely that if HAART is unable to completely restore MTB-specific immune responses and thereby reverse the risk of TB to levels present prior to acquisition of HIV infection, then the overall improvement in patient prognosis will result in an expanding survivor cohort with a chronically heightened susceptibility to TB. Thus, when considering the effect on TB control, the huge short-term benefit of HAART in reducing TB risk for the treated individual is unlikely to be reflected at the community level in the long term.

Williams and Dye (2003) modelled the potential impact of HAART on TB incidence, combining data on rates of CD4 cell decline during HIV infection, relative incidence of TB at different CD4 cell counts and the known impact of HAART on TB incidence [89]. They calculated that if HAART were given to patients with CD4 cell counts $< 200 \times 10^6$ cells/l with complete coverage and perfect compliance; the cumulative incidence of TB would be decreased by just 22% over 20 years. Conversely, to reduce TB incidence by 50% over 20 years with 85% effective coverage, use of HAART would have to be greatly expanded to include patients with CD4 cell counts $< 500 \times 10^6$ cells/l–an unrealistic proposition [89]. The small long-term impact of HAART on TB incidence is explained by the fact that HAART reduces the risk of TB but also extends life expectancy markedly. Thus, HAART-treated individuals develop TB at a lower rate but over a longer period of time.

The modelling data of Williams and Dye is based upon several assumptions. Calculations assume that HAART restores functional responses to MTB to levels present in the early stages of chronic HIV infection. However, at present there are insufficient data to know whether this is the case during long-term HAART. It is likely that there are gradual time-dependent changes in immune function, which are paralleled by time-dependent changes in patient susceptibility to TB. The modelled predictions are based on a 20-year time-scale whereas data on efficacy of HAART in reducing TB incidence are derived from studies with a much shorter duration of follow up. Moreover, the effect of HAART on rates of TB transmission and reinfection are not taken into account. Future data may help to further validate these modelling calculations.

The extent to which HAART restores long-term MTB-specific immune responses is critical and will be a key determinant of the extent to which HAART is able to contribute to TB control at the community level. Strategies that optimize MTB-specific immune restoration need to be explored. Current data indicate that early initiation of HAART may promote more complete immune restoration. Thus, in communities with high TB burden, early initiation of HAART may have an important long-term benefit in reducing TB rates. Further adjunctive strategies might also be explored to boost the restoration of MTB-specific immune responses. Such interventions might include use of bacille Calmette–Guerin vaccination during HAART or co-administration of cytokine therapy such as IL-2 [90]. Prophylaxis with isoniazid during HAART remains an alternative strategy.

The challenge of incident TB during HAART

Since HAART fails to reduce the incidence of TB to levels in HIV-negative individuals, a substantial rate of TB persists during HAART. This fact presents a major challenge to HAART programmes, especially in communities with a high prevalence of TB. In Cape Town, for example, our experience is that these problems are manyfold: (i) TB is an ongoing substantial cause of morbidity and mortality that consumes already over-stretched health resources; (ii) development of TB despite HAART undermines patient confidence in HAART; and (iii) concurrent treatment with HAART and anti-TB drugs presents difficulties associated with pill burden, patient adherence to treatment, pharmacokinetic interactions and overlapping toxicities [91]. An additional concern is the potential for nosocomial transmission of TB at HAART clinics; this is as yet unquantified. Thus, although the clinical benefits of HAART are undoubtedly huge, our experience at a successful community-based HAART project in Cape Town is that incident TB remains a major challenge among treated patients.

Conclusions

HAART has a remarkable impact on HIV/AIDS-associated morbidity and mortality and has greatly

Table 2. Summary of key points.

HAART reduces the incidence of TB by 70 to 90% in treated cohorts living in high and low TB incidence settings
HAART tends to restore cutaneous hypersensitivity responses to PPD and increases the proportion of patients who have typical post-primary radiological features of pulmonary disease
Immune reconstitution disease associated with TB reflects rapid and dysregulated restoration of TB-specific immunity in the first 3 months of HAART
HAART restores both the quantity and function of antigen-specific T cells. However, even among the small proportion of patients who normalize their CD4 cell counts, phenotypic and functional defects persist
The greater the degree of immunodeficiency at the time HAART is initiated, the more limited the extent of immune restoration that is possible
The incidence of TB during HAART remains higher than that among HIV-noninfected individuals
HAART is unlikely to play a prominent role as a TB control measure. Incomplete immune restoration and greatly increased longevity during HAART means that patients will have a chronically heightened TB risk over a greatly increased life-span
Strategies to optimize restoration of TB-specific immune function during HAART need to be explored. Identification and treatment of patients before they develop advanced immunodeficiency may be critical in this respect

HAART, Highly active antiretroviral therapy; TB, tuberculosis; PPD, purified protein derivative.

extended the prognosis of people living with the virus. We have reviewed how suppression of viral replication permits gradual restoration of lymphocyte numbers, phenotype and function over many years. The extent to which CD4 cell counts are restored is variable. Current data, however, show that even among those patients who develop full numeric restoration of circulating CD4 cell lymphocytes, phenotypic abnormalities and functional defects persist. Persistent abnormalities are most likely when immunodeficiency is more advanced at the time of initiation of HAART (Table 2).

Long-term deficits in MTB-specific immune responses are likely to be an important factor contributing to the higher than expected incidence of TB among patients receiving HAART. Suboptimal restoration of MTB-specific immunity hinders the extent to which HAART may play a role in TB control at the community level since patients receiving HAART survive longer and yet maintain a chronically heightened risk of TB. Thus, although the benefit of HAART to the individual in reducing TB risk is great, the impact at the community level is much less. Current modelling analysis predicts that HAART will have a more limited impact on TB incidence than might have been anticipated (Table 2).

Future epidemiological studies need to more clearly define the long-term incidence of TB during HAART in prospective study cohorts in low-income countries. The impact of HAART on TB epidemiology at the community level should also be studied, using careful TB surveillance to determine the effect on both the burden of disease and patterns of TB transmission within the community. The extent to which transmission of TB occurs at antiretroviral clinics also needs to be assessed. Laboratory studies should prospectively define the rate and extent to which functional immune responses to TB are restored during long-term HAART. This would permit factors associated with suboptimal restoration to be identified and strategies to augment MTB-specific immune responses to be explored. The timing of initiation of HAART may prove to be the most important factor in this respect and early initiation of HAART may lead to long-term benefits in TB risk reduction. However, widespread early initiation of HAART in low-income countries would be very costly in health resources and detailed cost-benefit analyses are required. Nevertheless, as access to HAART expands in low-income countries and treatment recommendations are established and refined, the long-term effect of HAART on TB control needs to be carefully evaluated.

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Correspondence

Risk Factors for Tuberculosis among HIV-infected Patients Receiving Antiretroviral Treatment

To the Editor:

Although tuberculosis (TB) incidence among HIV-infected cohorts receiving highly active antiretroviral treatment (HAART) is reduced by 70–90%, it remains substantially higher than among HIV-noninfected patients (1, 2). Strategies to reduce the burden of TB during HAART are needed. We therefore read with interest the article by Seyler and colleagues, which reported risk factors for TB among patients receiving HAART in Abidjan (3).

Using multivariate analysis, Seyler and colleagues found that a past history of TB was the only significant risk factor for TB during HAART (3). The 31 patients in the cohort with a past history of TB had all been declared cured following 6 mo or more of rifampicin-containing anti-TB treatment, and yet six developed TB during HAART over a median of 26 mo observation. This represents an unusually high recurrence rate (4) even without taking into account the major protective effect of HAART. Of note, however, 33% of the incident cases were smear- and culture-negative; potential misdiagnoses of active TB among patients with symptomatic posttuberculous lung disease may have confounded past history of TB as a risk factor.

Advanced immunodeficiency might be expected to be associated with an ongoing increased risk of TB during HAART, at least until a satisfactory degree of immune restoration has been achieved. We have studied a large cohort in Cape Town (described in Reference 2) that includes patients with a diverse range of CD4 cell counts and clinical stages. Analysis reveals that the ongoing risk of TB during HAART is much greater among those with low nadir CD4 cell counts and advanced clinical stage of disease; past history of TB is not a risk factor. The disparities with the findings of Seyler and colleagues may reflect differences in diagnostic criteria for TB and the fact that the restricted enrollment criteria for the Abidjan cohort resulted in inclusion of few patients with stage 1 or 2 disease or CD4 cell counts greater than 200 cells/μl; the resulting cohort composition diminishes the power to assess CD4 count and clinical stage as risk factors. This is a problem common to analysis of data from many cohorts receiving HAART in Africa where often only those with advanced disease are treated.

If it is confirmed that the risk of TB during HAART is associated with advanced pretreatment immunodeficiency, the risk might be decreased by earlier initiation of HAART. In addition, those patients with advanced immunodeficiency may derive greatest benefit from adjunctive treatments such as isoniazid prophylaxis.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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From the Authors:

Lawn and colleagues raise a number of crucial points. However, we disagree with them on most of these issues. They state that, in our study, the rate of tuberculosis (TB) in patients with a past history of TB (11.3/100 person-years) represents an "unusually high recurrence rate." In a recent report by Grant and coworkers (1), the incidence of TB in non–HAART-treated South African adults with a past history of TB was estimated at 19.3/100 person-years. In Grant and coworkers' study, as in ours, the "rate of TB in persons with a past history of TB" is not a "recurrence rate" but the rate of TB after the date of study enrollment, which was not the date of the end of the previous TB episode. In our patients with a history of TB, the mean time since the last TB episode was 2.8 yr. Including this time in the period at risk would obviously have led to an estimate of recurrence rate quite lower than the TB rate after enrollment.

In the study by Grant and coworkers, the rate of TB in non–HAART-treated patients with no TB history was half that of patients with TB history (1). We observed the same phenomenon in patients on HAART. Lawn and colleagues report that they did not, although they do not provide sufficient data to allow us to comment on the strength and limits of their analysis. We would be interested in seeing their data on TB incidence in patients with and without TB history (even if not significantly different in their sample), and in the procedures used to document a TB history at study entry.

Documenting smear- and culture-negative (SCN) TB requires standardized diagnosis criteria and systematic review by an event documentation committee. Both conditions were applied in our study. In HIV-infected patients, SCN TB has long been recognized as a frequent problem, even in industrialized countries (2, 3). In reports from sub-Saharan settings, the percentage of SCN episodes which are diagnosed as "possible TB" by experienced infectious diseases specialists is frequently high (4). Where low rates are reported, one expects that patients with SCN TB died before being diagnosed. SCN TB is frequently extrapulmonary and found in patients with low CD4 count. Therefore, SCN TB and culture-positive TB may be associated with different risk factors. Contrary to Lawn and colleagues, we don't believe that SCN TB should be excluded from TB risk factor studies.

Finally, we agree with Lawn and colleagues on one point: studies performed in sub-Saharan Africa frequently include patients with low CD4 count. Some of them occasionally give negative conclusions on patients with high CD4 count, despite insufficient power (5). In our reduced sample of patients with low CD4 count, CD4 was not significantly associated with TB

Incidence of Tuberculosis during Highly Active Antiretroviral Therapy in High-Income and Low-Income Countries

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(See the article by The Antiretroviral Therapy Cohort Collaboration on pages 1772–82)

Highly active antiretroviral therapy (HAART) has revolutionized the care of HIV-infected individuals, causing major reductions in HIV-associated morbidity and mortality. Once blood $CD4^+$ cell counts have reached stable levels of >200 cells/μL, the risk of developing opportunistic disease due to cytomegalovirus, *Pneumocystis jirovecii, Mycobacterium avium* complex (MAC), *Toxoplasma gondii,* and *Cryptococcus neoformans* is generally very low [1]. This reflects the fact that partial restoration of the immune system is sufficient to suppress these low-virulence pathogens. In contrast, however, it is emerging that patients receiving HAART retain a chronically heightened risk of disease due to other, more virulent pathogens, such as tuberculosis (TB) and invasive pneumococcal disease [2–4]. In the case of TB, it might be hypothesized that this observation might relate to the coexistence of other risk factors for TB or possibly to nosocomial TB exposure at HIV treatment facilities. However, a principal underlying cause is undoubtedly the persistence of deficits in immune function during treatment [5]. Because the risk of TB is increased even among those with minor degrees of HIV-associated immunodeficiency, complete normalization of immune function during HAART would be required to reduce the risk of TB to background levels. Increasing evidence, however, shows that this goal is generally not attainable. Even among patients who have good responses to HAART, functional immunological deficits usually persist [5–7], including those specific to *Mycobacterium tuberculosis* [5, 8, 9].

Despite limitations in the extent to which TB-specific immunity may be restored [5], HAART nevertheless has a major impact on TB incidence in treated cohorts. Studies conducted in both countries with a high prevalence of TB and those with a low prevalence of TB have shown that HAART reduces the risk of TB by 70%–90% [3, 10–14]. The duration of follow-up in these studies was limited, however; therefore, these results may not reflect the full beneficial effects to be derived from HAART in the longer term.

The article by Girardi and colleagues [15] in this issue of *Clinical Infectious Diseases* reports the burden of TB during 3 years of HAART in cohorts in Europe and North America. In high-income countries in which TB prevalence is low, such a study could only be achieved by collaborative analysis of data sets from multiple cohorts—a major strength of this article. A key finding was that HAART resulted in time-dependent reductions in TB incidence throughout follow-up. Although the greatest reduction was during the first year of treatment, the rate continued to decrease, with a ~5-fold reduction between the first and third years (figure 1). These changes are likely to reflect incremental gains in $CD4^+$ cell count and function, which are greatest during the first 1–2 years of HAART, with only small additional increases occurring thereafter [16]. It remains an open question as to whether TB incidence rates may decrease further during long-term HAART and whether background rates are ultimately reached. In the study by Girardi and colleagues [15], the incidence at 3 years was still several-fold higher than the rates in the general population where these patients were treated. However, extrapolation of these data (figure 1) would suggest that any further decreases in TB incidence beyond 3 years are likely to be small. Moreover, it is actually impossible to define the background TB incidence rate for these cohorts, because the individuals included may differ from the general population in having other long-term risk factors for TB.

The TB incidence rate reported by Gir-

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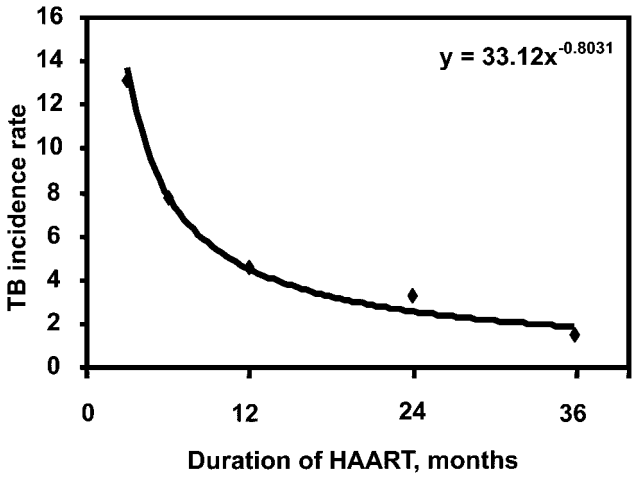

Figure 1. Changes in tuberculosis (TB) incidence during 3 years of HAART with regression curve fitted. TB incidence rate is expressed as number of cases per 1000 person-years of follow-up. Data are adapted from the study by the Antiretroviral Therapy Cohort Collaboration [15].

ardi and colleagues [15] was particularly high during the first 3 months of HAART. Several different reasons may underlie the presentation of cases during this interval: 1) many cases will have arisen at this time because of residual immunodeficiency, 2) intensive investigation of some patients with symptomatic disease during preparation for HAART may have yielded results that only became positive after HAART initiation, and 3) some cases of previously subclinical disease may have manifested as immune reconstitution disease [17], as has been reported by one of the centers collaborating in this study [18].

A substantial weakness of the study by Girardi and colleagues [15] was that patients with AIDS were excluded; such patients are the very individuals who have the greatest risk of TB [3]. Thus, the TB incidence rates reported in this study do not reflect the overall burden of HIV-associated TB in these cohorts. Moreover, because the extent of restoration of TB-specific immunity during HAART is limited, particularly among those with AIDS [5, 8], the long-term rates of TB among such patients are likely to be higher than those reported in this study.

Girardi and colleagues [15] identified risk factors for TB during HAART using multivariate analysis. The association of TB risk with route of HIV acquisition is likely to be attributable to the association of this variable with country of origin or social factors, which are also risk factors for TB. Unfortunately, the ethnic origin of patients was not recorded. The strongest risk factor for AIDS-associated TB in London is African origin [19], and this increased risk is likely to persist during HAART. Confirmation of this might permit targeting of chemoprophylaxis to further reduce TB incidence among such high-risk groups.

Girardi and colleagues [15] also identified low baseline $CD4^+$ cell count as an independent risk factor for the development of TB; in addition, risk of TB occurring after 6 months of HAART was associated, not only with baseline $CD4^+$ cell count, but also with the $CD4^+$ cell count at 6 months after initiation of HAART. With increasing duration of HAART, it might be hypothesized that the risk of TB would become more strongly associated with current $CD4^+$ cell count and less strongly associated with baseline $CD4^+$ cell count. Alternatively, because long-term failure of immune functional recovery is particularly associated with advanced pretreatment immunodeficiency [7, 8], it is possible that, among those with advanced disease, a low baseline $CD4^+$ cell count may actually predetermine a long-term increased risk of TB.

Access to HAART is now rapidly expanding in low-income countries where the prevalence of TB is high and TB is the predominant cause of HIV-associated morbidity and mortality [20]. In a cohort based in a hospital out patient service in Cape Town, South Africa, the TB incidence was 24 cases per 1000 person-years during a median of 16 months of follow-up [3]; after >3 years of follow-up, this rate was ~10 cases per 1000 person-years (unpublished data), which is ~7-fold higher than the rate found in the study by Girardi and colleagues [15]. The burden of TB during HAART is even greater within a local community-based antiretroviral program in Gugulethu (near Cape Town, South Africa), where the annual TB incidence in the general community exceeds 1000 cases per 100,000 population [21]. Among HIV-infected patients receiving HAART at that location, 10%–15% develop TB during the first year of HAART (unpublished data). This huge burden of disease has great implications in terms of morbidity, utilization of health resources, and difficulties associated with concurrent administration of both HAART and antituberculosis treatment. These difficulties relate to pill burden, patient adherence, pharmacokinetic interactions, and overlapping drug toxicities.

The implications go further. Because the TB incidence remains much higher among patients receiving HAART than among the general community, modelling analysis suggests that good, effective coverage with HAART may have relatively little impact on the overall burden of TB in communities in low-income countries over a 20-year time span [22]. The reason for this is that life expectancy is greatly extended during HAART, and therefore, although the risk of TB per unit time for individuals receiving HAART is greatly reduced, the lifetime risk of disease may not decrease [5, 22]. Background data for this modelling analysis, however, were derived from a cohort with a short duration of

follow-up, and longer term studies of the effect of HAART on incidence rates and risk factors for TB in low-income countries are needed.

A recently published study from Abidjan found that past history of TB was the sole risk factor for development of TB during HAART [23]. This article, however, has limitations related to the size of the study population, the number of TB cases, restricted cohort composition, and diagnostic criteria for TB, potentially confounding the findings [23, 24]. The study by Girardi and colleagues [15] excluded patients who received an AIDS diagnosis before initiation of HAART, thereby excluding those with a past history of TB and precluding examination of this variable as a risk factor. However, our findings in Cape Town, South Africa, agree with those of Girardi and colleagues [15]. We have found, in a prospective cohort study, that risk of TB was strongly associated with the pretreatment level of immunodeficiency, as reflected by the baseline $CD4^+$ cell count and clinical stage of disease (as defined by the World Health Organization); past history of TB was not a risk factor [24]. Moreover, we also found that the immunological response to HAART was impaired among patients who developed TB, compared with those who remained free of TB (unpublished data), suggesting that poor treatment response was an important underlying mechanism. Again, this agrees with the findings of Girardi and colleagues [15].

Collectively, these data demonstrate that during the first 3 years of HAART, in both high- and low-income settings, risk of TB is associated with the baseline level of immunodeficiency. This has important implications for the antiretroviral treatment programs in low-income countries. The median blood $CD4^+$ cell count among patients enrolling in our own community-based antiretroviral program in Cape Town is <100 cells/μL, as is typically the case among programs elsewhere in sub-Saharan Africa [25]. As a result of diminished capacity for immune recovery, initiation of HAART among patients with advanced disease may result in an expanding cohort of patients who remain vulnerable to TB. This may undermine the potential benefits of HAART in TB control, and barriers to earlier access to HAART in these settings need to be overcome.

In conclusion, despite major reductions in TB incidence among individuals receiving HAART, TB risk remains elevated among those receiving treatment in both high- and low-income countries. This ongoing burden of disease has major implications for antiretroviral programs in low-income countries and undermines the potential for HAART to contribute to TB control at the community level.

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Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort

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Objectives: To determine the long-term incidence of tuberculosis (TB) and associated risk factors among individuals receiving HAART in South Africa.

Design: Prospective cohort study.

Methods: Microbiologically or histologically confirmed incident TB was identified in a hospital-based cohort of 346 patients receiving HAART between 1996 and 2005 in Cape Town.

Results: The TB incidence density rate was 3.5/100 person-years in the first year and significantly decreased during follow-up, reaching 1.01/100 person-years in the fifth year ($P = 0.002$ for trend). TB incidence during the study was highest among patients with baseline CD4 cell counts < 100 cells/μl and those with World Health Organization (WHO) clinical stage 3 or 4 disease (5.71 and 3.88/100 person-years, respectively). Risk of TB was independently associated with CD4 cell count < 100 cells/μl (adjusted risk ratio [ARR], 2.38; 95% confidence interval (CI), 1.01–5.60; $P = 0.04$), WHO stage 3 or 4 disease (ARR, 3.60; 95% CI, 1.32–9.80; $P = 0.01$) and age < 33 years (ARR, 2.86; 95% CI, 1.29–6.34; $P = 0.01$). Risk of TB was not independently associated with plasma viral load, previous history of TB, low socioeconomic status or sex. Despite similar virological responses to HAART, blood CD4 cell count increases were much smaller among patients who developed TB than among those who remained free of TB.

Conclusions: Incidence of TB continues to decrease during the first 5 years of HAART and so HAART may contribute more to TB control in low-income countries than was previously estimated from short-term follow-up. Patients with advanced pretreatment immunodeficiency had persistently increased risk of TB during HAART; this may reflect limited capacity for immune restoration among such patients.

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Keywords: antiretroviral treatment, HAART, tuberculosis, HIV, Africa

Introduction

Use of HAART has dramatically decreased HIV-associated morbidity and mortality in many high-income countries since the mid 1990s [1–4]. Suppression of viral replication permits both quantitative and functional reconstitution of the immune system [5,6]. As a result, primary and secondary prophylaxis for many opportunistic infections that occur among patients with advanced immunodeficiency can subsequently be discontinued [7–10]. The risk of recurrence of these low virulence opportunistic pathogens, including cytomegalovirus, *Pneumocystis jirovecii*, *Mycobacterium avium* complex, *Toxoplasma gondii* and *Cryptococcus neoformans* is generally very low once blood CD4 cell counts have reached stable levels > 200 cells/μl [7].

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Studies conducted in countries with either low or high TB prevalence have also shown that TB incidence is reduced by 70–90% in HIV-infected cohorts receiving HAART [11–16]. However, in contrast to low-virulence pathogens, increasing evidence suggests that significant rates of TB persist during HAART [16–18]. Therefore, although the risk of TB is greatly diminished during treatment, the risk nevertheless remains substantially higher than that among individuals who do not have HIV. This has important implications for the extent to which HAART may assist in TB control in low-income countries [19]. Modelling analysis suggests that good effective coverage with HAART in such communities may have relatively little impact on the overall burden of TB over a 20-year timespan [20]. The reason for this is that life expectancy is greatly extended during HAART and so treated patients may develop TB at a lower rate but over a much longer period of time [19,20]. However, these existing analyses were based upon data from a cohort with a short median duration of follow-up [16] and the longer-term effect of HAART on TB incidence rates remains undefined.

The existing TB control strategy of case-finding and directly observed treatment of sputum smear-positive patients using short-course antituberculosis treatment is proving inadequate in countries with a high burden of HIV [21,22]. The World Health Organization (WHO) has, therefore, formulated a strategic framework aimed at functional integration of control programmes for TB and HIV/AIDS [23]. HAART is one element within this framework but data are now needed to clarify the effect that HAART will have on TB control. This study determined TB incidence rates during HAART and identified risk factors for TB in a treatment cohort studied in Cape Town, South Africa, between 1996 and 2005.

Methods

Study population

The study was based on the Cape Town AIDS Cohort, which has been described in detail previously [16,24]. Patients were enrolled into this cohort from 1996 onwards when HAART was not available in the public sector in South Africa. These patients, therefore, accessed HAART through participation in multicentre phase III clinical trials at the New Somerset Hospital and the Desmond Tutu HIV Research Centre, University of Cape Town. Participants gave informed consent and clinical trials protocols were approved by the University of Cape Town Clinical Research Ethics Committee.

Enrolment criteria for HAART differed between the various trials but collectively encompassed patients with a wide spectrum of baseline blood CD4 cell counts and clinical stages [16]. Patients were clinically staged using the WHO criteria. Demographic data were recorded, including socioeconomic status according to the Cape Metropolitan Council suburbs composite index, as described previously [16]. At baseline and at each follow-up time-point, patients were clinically assessed and symptomatic disease was investigated. All patients received at least three antiretroviral drugs: a non-nucleoside reverse transcriptase inhibitor and two nucleoside reverse transcriptase inhibitors; or three nucleoside reverse transcriptase inhibitors; or a protease inhibitor with two nucleoside reverse transcriptase inhibitors. Patients were reviewed routinely every 2–3 months or more frequently if clinically indicated. Blood CD4 cell counts and plasma viral load were measured every 2–3 months. Systematic clinical records were maintained and were used to update a prospective electronic database. Patients were excluded from the analysis if they were receiving TB treatment at enrolment.

Case definition for tuberculosis

TB was defined as either *definite* – culture of *Mycobacterium tuberculosis* or a postmortem diagnosis of active TB – or *presumptive* – acid-fast bacilli present in sputum or tissue samples or caseating granulomata seen in histological samples obtained from a patient with a clinical presentation consistent with TB and who subsequently responded to antituberculosis treatment.

Statistical analysis

Differences in proportions were compared by χ^2 test. Intraindividual paired comparisons of median CD4 cell counts and plasma HIV RNA concentrations were carried out using the Wilcoxon signed rank test. The Mann–Whitney *U* test was used to compare these values between different groups of subjects. Trend analyses were conducted using Cochrane–Armitage test for linear trend. All tests were two sided and a *P* value of 0.05 was considered significant.

TB incidence density rate (IDR) was defined as the number of new episodes of TB occurring per 100 patient-years of observation. The analysis was further stratified by the baseline HIV RNA viral load (< 5 or ≥ 5 $\log_{10}$ copies/ml), CD4 cell count (< 100 or ≥ 100 cells/μl), WHO clinical stage (stage 3 or 4 versus 1 or 2), socioeconomic status, previous history of TB, age (above or at/below median age), and gender. Different CD4 cell count categories were modelled, but a cut-off of 100 cells/μl was used in the final analysis because it was significantly associated with risk of TB in this cohort.

Kaplan–Meier plots were used for TB-free survival probabilities. TB-free survival was defined as the time from enrolment to the date of TB diagnosis, death from any cause or the last follow-up visit. TB-free survival was stratified further by baseline immunological and clinical

status and was compared using the generalized log rank test. Univariate and multivariate Cox proportional hazards regression models were fitted to determine the risk of TB, which was expressed as a rate ratio. Variables were considered for inclusion into the multivariate model if they were found associated with the risk of TB in the univariate analyses at $P < 0.15$. Past history of TB was included *a priori* in view of previously published findings [17]. The assumption of proportional hazards was examined by plotting the log[−log(survival function)] estimates against log time plots. When determining the effect of response to HAART on risk of TB, blood CD4 cell count and plasma viral load measurements made within the 3 months prior to TB diagnoses were used. PEPI version 4.0 (Sagebrush Press, Salt Lake City, Utah, USA), STATISTICA release 6.6 (Tulsa, Texas, USA) and SAS version 8.2 (SAS, Cary, North Carolina, USA) software were used for data analysis.

Results

Patients and follow-up

Between 1996 and 2005, 363 patients received HAART. At enrolment, 17 patients were receiving TB treatment and were, therefore, excluded from the analysis. At baseline, the remaining 346 patients had a median age of 33 years [interquartile range (IQR), 28–40], 190 (55%) were male and 178 (51%) were of low socioeconomic status. Their median blood CD4 cell count was 242 cells/μl (IQR, 120–343); median plasma viral load was 4.9 $\log_{10}$ copies/ml (IQR, 4.4–5.5); 178 (51%) had symptomatic disease (WHO clinical stages 3 or 4); and 47 (14%) had a previous episode of TB that had been diagnosed a median of 13 months (IQR, 9–23) prior to enrolment.

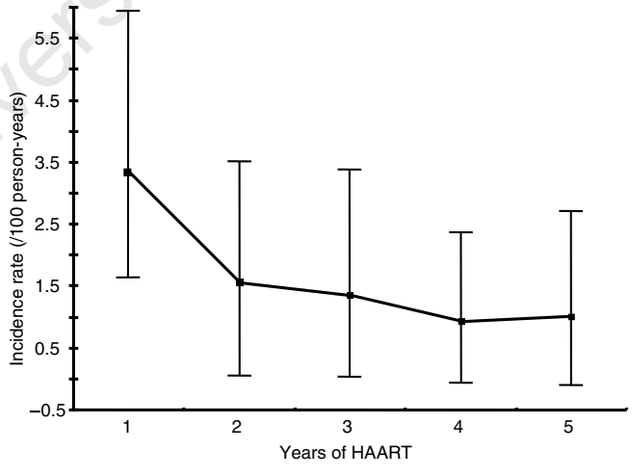
The median duration of follow-up was 40 months (IQR, 18–53; range, 0.78–104). During a total of 1108 person-years of observation, 27 cases of TB were diagnosed. Of these, 20 were presumptive and 7 definite; 22 were pulmonary and 5 were extrapulmonary. Of 30 deaths within the cohort, three (10%) were among those who were diagnosed with TB.

TB incidence rates

All 27 episodes of TB occurred within the first 5 years of follow-up and the overall TB IDR was 2.44/100 person-years [95% confidence interval (CI), 1.61–3.54]. A significant reduction in IDR was observed over the 5-year period, decreasing from 3.35/100 person-years in the first year to 1.01/100 person-years in the fifth year (Fig. 1). It was possible that this decrease resulted from a changing cohort composition during follow-up. However, trend analysis revealed that the proportion of patients remaining in the cohort from year to year did not differ according the risk factors identified in subsequent analysis: WHO clinical stage ($P = 0.41$ for trend), baseline CD4 cell count < 100 cells/μl ($P = 0.24$ for trend) and age ($P = 0.07$ for trend).

Baseline characteristics and risk of tuberculosis

TB incidence rates were calculated for patients stratified by baseline characteristics (Table 1). This analysis showed



No. patients at risk	346	294	238	201	112
No. with tuberculosis	11	5	4	4	3
Incidence (/100 person-years) (95% CI)	3.35 (1.67–5.98)	1.56 (0.51–3.65)	1.36 (0.37–3.47)	0.9 (0.26–2.40)	1.01 (0.21-2.96)

Fig. 1. Tuberculosis incidence density rates. $P = 0.02$ for trend; slope $y = -0.52x + 3.23$; $R^2 = 0.72$). CI, confidence interval.

Table 1. Tuberculosis incidence density rate stratified by baseline sociodemographic and clinical characteristics.

Characteristics	No. of patients	Person-years	No. with TB	TB IDR (95% CI)[a]	*P* value[b]
Total Patients	346	1108.8	27	2.44 (1.61–3.54)	
Age (years)					
< 33[c]	162	475.5	18	3.79 (2.25–5.98)	
≥ 33	184	633.2	9	1.42 (0.65–2.70)	< 0.01
Sex					
Male	190	632.1	12	1.90 (0.98–3.31)	
Female	156	476.7	15	3.15 (1.76–5.19)	0.19
Socioeconomic status					
Low	178	650.6	17	3.03 (1.77–4.86)	
High	168	548.2	10	1.82 (0.88–3.36)	0.36
Past history of TB					
Yes	47	155.4	3	1.93 (0.39–5.64)	
No	299	953.5	24	2.52 (1.61–3.74)	0.64
CD4 cell count (cells/μl)					
≥ 100	273	898.8	15	1.67 (0.93–2.75)	
< 100	73	210.1	12	5.71 (2.95–9.96)	< 0.0001
Viral load ($\log_{10}$ copies/ml)					
< 5	199	633.1	10	1.58 (0.76–2.90)	
≥ 5	147	475.9	17	3.57 (2.14–5.72)	0.04
WHO clinical stage					
Stage1 or 2	168	567.4	6	1.06 (0.39–2.30)	
Stage3 or 4	178	541.4	21	3.88 (2.40–5.98)	0.003

TB, tuberculosis; IDR, incidence density rate (per 100 person-years); CI, confidence interval.
[b]Obtained by χ^2 test for difference in TB IDR.
[c]Median age of the cohort.

significantly higher incidence rates among those with the following characteristics: age < 33 years (median age of the cohort), nadir blood CD4 cell counts < 100 cells/μl, baseline plasma viral load ≥ 5 log copies/ml and symptomatic (WHO stage 3 and 4) disease at enrolment. Incidence rates did not differ according to sex, socioeconomic status or previous history of TB.

The TB-free survival proportion for the total cohort over the 5-year follow-up period was 0.88 (Fig. 2a). However, the 5-year TB-free survival proportion was significantly lower among patients with baseline CD4 cell count < 100 cells/μl than in those with ≥ 100 cells/μl (81% versus 92%; Fig. 2b). Similarly, the survival proportion was lower among patients with baseline WHO stage 3 or 4 compared with those with WHO stage 1 or 2 (81% versus 94%; Fig. 2c). The lowest 5-year TB-free survival proportion was among patients with WHO stage 3 or 4 disease and who also had a CD4 cell count < 100 cells/μl (Fig. 2d). Of the total TB episodes occurring among this latter subgroup, 50% developed during the first 6 months of HAART.

Cox regression analyses were used to analyse risk factors for TB and showed that the risk of TB was independently associated with a baseline CD4 cell count < 100 cells/μl, WHO clinical stage 3 or 4 and age < 33 years (Table 2). Past history of TB was included in the multivariate analysis *a priori* and, although it was not associated with risk of TB in the univariate analysis, there was a trend in multivariate analysis towards a past history having a protective effect against TB. A subanalysis assessing risk factors associated with development of TB during the first year of ART alone showed that the single variable independently associated with risk of TB was a baseline blood CD4 cell count < 100 cells/μl (adjusted risk ratio, 6.14; 95% CI, 1.44–26.01; $P = 0.01$).

Tuberculosis and response to HAART

Having determined the effect of baseline patient characteristics on risk of TB, a further analysis examined whether the virological and immunological responses to HAART were also associated with risk of TB. Among the 27 patients who developed TB, the median plasma viral load at the time TB was diagnosed was 2.98 $\log_{10}$ copies/ml (IQR, 2.60–4.16), which was significantly lower than that at enrolment (5.38 $\log_{10}$ copies/ml; IQR, 4.69–5.87) ($P < 0.0001$; Fig. 3a). Furthermore, the median blood CD4 cell count at the time of TB diagnosis (198 cells/μl; IQR, 79–397) was significantly higher than at baseline (112 cells/μl; IQR, 58–253) ($P = 0.025$) (Fig. 3b). These data indicated that those who developed TB had responded virologically and immunologically to HAART at the time of TB diagnosis. However, although the rates of viral load suppression did not differ significantly between those patients who developed TB and those who remained free of TB (Fig. 3c), the immunological responses of the two groups differed markedly. The median increase in blood CD4 cell count among those who developed TB (74 cells/μl; IQR, 70–76) was significantly smaller than that of patients who remained free of TB (248 cells/μl; IQR, 185–312) ($P = 0.007$; Fig. 3d). Therefore, TB developed among patients whose immunological responses to HAART were suboptimal.

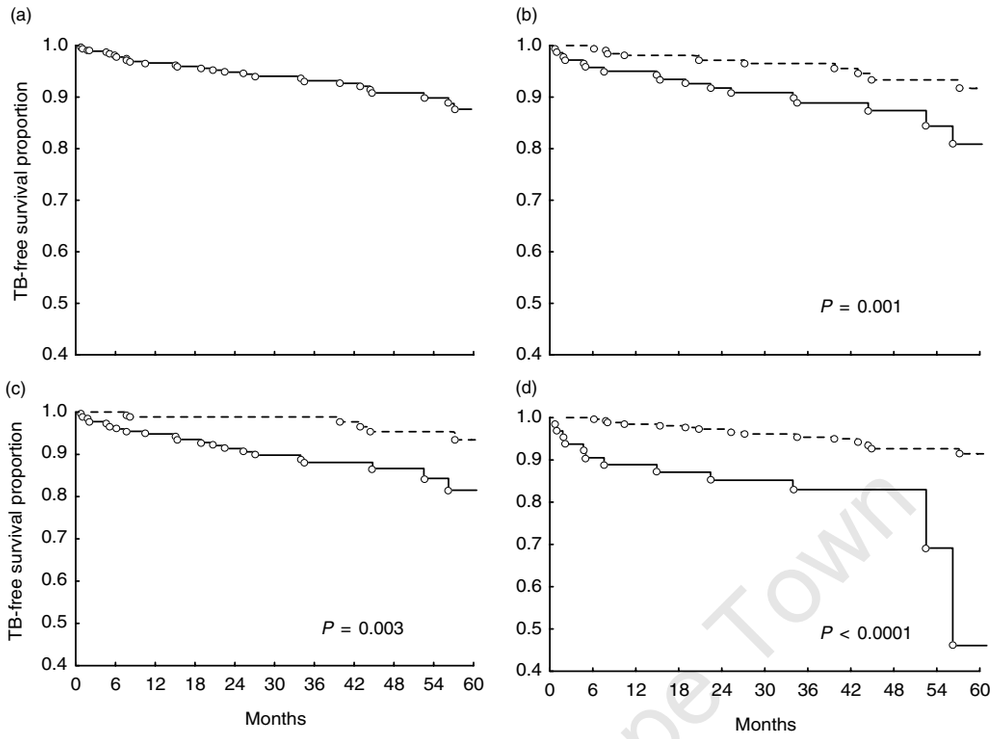


Fig. 2. Kaplan–Meier plots of tuberculosis (TB)-free survival proportion. (a) In the total cohort; (b) among patients stratified by baseline CD4 cell count: - - -, > 100 cells/μl; ——, < 100 cells/μl); (c) among patients stratified by WHO clinical stage of disease: - - -, - - -, WHO stage 1 or 2; ——, WHO stage 3 or 4; (d) among patients with baseline CD4 cell count < 100 cells/μl and WHO stage 3 or 4 (——) compared with the remainder of the cohort (- - -). *P* values from the log rank test are given.

Discussion

This study documented the incidence rates and risk factors for TB in a South African cohort of patients receiving HAART over a median duration of follow-up of 40 months. Previous studies in low-income countries have been of much shorter follow-up and have not documented time-dependent changes in TB incidence rates during HAART [15–17]. A further important strength of this cohort is that broad enrolment criteria for

Table 2. Cox regression analyses of predictors of tuberculosis in the whole cohort.

	Univariate analysis		Multivariate analysis	
Variable	RR, (95% CI)	*P* value[a]	ARR, (95% CI)	*P* value[a]
Age (years)				
< 33[b]	2.23 (1.02–4.88)	0.04	2.86 (1.29–6.34)	0.01
≥ 33	1		1	
Past history of tuberculosis				
Yes	0.76 (0.23–2.53)	0.66	0.31 (0.09–1.08)	0.07
No	1		1	
CD4 cell count (cells/μl)				
≥ 100	1		1	
< 100	3.50 (1.62–7.56)	0.001	2.38 (1.01–5.60)	0.04
Viral load (log_{10} copies/ml)	1.74 (1.03–2.94)	0.04	1.28 (0.72–2.29)	0.40
WHO clinical stage				
Stage 1 or 2	1		1	
Stage 3 or 4	3.72(1.50–9.26)	0.005	3.60 (1.32–9.82)	0.01

RR, rate ratio; ARR, adjusted rate ratio; CI, confidence interval.
[a]Obtained by χ^2 test for difference in tuberculosis incidence density rate (per 100 person-years)
[b]Median age of the cohort.

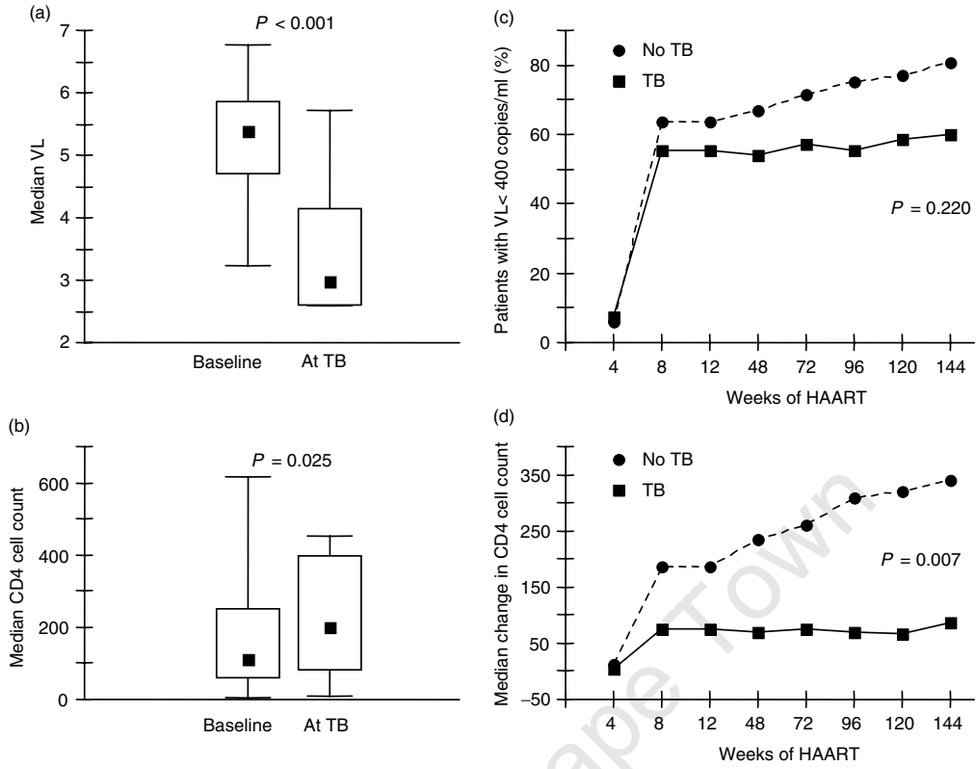


Fig. 3. Changes in plasma viral load (VL; log copies/ml) and blood CD4 cell counts (cells/μl) during HAART. Among patients who developed tuberculosis (TB; $n = 27$), median plasma VL at baseline was compared with that at the time TB was diagnosed (a), and the blood CD4 cell count at baseline was compared with that at the time of TB diagnosis (b). Box and whisker plots show the median (square), 25th and 75th centiles (box) and range (whiskers). (c) The percentage of patients with plasma VL suppressed to < 400 copies/ml during follow-up comparing those who remained free of TB with those who developed TB. (d) The median change in blood CD4 cell count during follow-up from baseline, comparing patients who remained free from TB with those who developed TB.

HAART resulted in a cohort with diverse baseline patient characteristics, including a wide spectrum of baseline blood CD4 cell counts and WHO clinical stages of disease. This increased the power of the analysis to identify risk factors for TB. All diagnoses were supported by microbiological or histological evidence, further strengthening the data. However, the low event frequency beyond 5 years of follow-up prevented estimation of TB incidence rates beyond this time-point, and a larger cohort would be needed to examine this. Furthermore, data from this hospital-based cohort may not reflect TB incidence rates observed in community-based HAART programmes.

We have previously reported from this same cohort that HAART reduces the incidence of TB by approximately 80%; however, the mean duration of HAART in this previous analysis was 16.8 months [16]. Data in the present study showed an ongoing time-dependent reduction in TB incidence, which was more than three-fold lower in the third year of HAART compared with the first year. These data have implications for mathematical modelling calculations that assume that HAART is likely to have limited impact on the TB incidence at the community level in low-income countries [20]. The previously reported TB incidence rate in this cohort was 2.4/100 person-years over a mean of 16.8 months receiving HAART [16]; however, the present data show that the rate further decreased to approximately 1.0/100 person-years after 5 years of treatment. These new data suggest that the likely long-term impact of HAART on the community burden of TB may be greater than previously estimated.

The decreases in TB incidence rates observed in this study are likely to reflect time-dependent changes in TB-specific immune function. The extent of immune reconstitution resulting from HAART is greatest during the first 2 years of treatment, with small further increments in CD4 cell numbers occurring in the longer term among some patients [19]. Such a pattern was mirrored by the reductions TB incidence rates observed in this study (Fig. 1). However, increasing evidence suggests that long-term restoration of immune cell

phenotype and function are limited, even among the minority of patients whose blood CD4 cell counts normalize [19,25,26]. From an immunological perspective, it is, therefore, unlikely that TB risk during long-term HAART will return to the levels seen among individuals who do not have HIV infection. An alternative explanation for the reduction in TB incidence rates during follow-up, the possibility that an element of survival bias may have resulted from deaths during follow-up ($n = 30$), cannot be excluded. However, the majority of deaths occurred early after initiation of treatment and could not account for ongoing reduction in TB rates over several years. Moreover, analysis of cohort composition during follow-up did not reveal significant changes in the proportion of patients who had identified risk factors for TB.

We have shown in this study that age < 33 years (median age of the cohort), baseline blood CD4 cell count (< 100 cells/μl) and WHO clinical stages 3 and 4 were all independently associated with a increased risk of TB. It is unclear why younger age was a risk factor, but this may possibly reflect behavioural differences that affect exposure. In the analysis of baseline blood CD4 cell count as a risk factor, categorization of patients using a cut-off of 100 cells/μl represented the best-fit for regression analyses but does not necessarily represent a risk threshold. Risk factors for TB found in this study contrast markedly with the findings of a study from West Africa [17]. In this study, past history of TB was the only risk factor for development of TB during HAART. However, the study had design limitations related to the size of the study population, the number of TB cases, diagnostic criteria for TB and restricted cohort composition [27]. In settings with high TB incidence but low rates of multidrug resistance, a 6-month course of antituberculosis treatment containing rifampin might rather be expected to confer a time-limited reduction in TB risk among HIV-infected individuals, as was suggested by the trend seen in the multivariate analysis in the present study.

Among HIV-infected individuals who are not receiving HAART, risk of TB increases with increasing HIV-associated immunodeficiency [28]. The data presented here indicate that those with the most advanced pretreatment immunodeficiency (as shown by both blood CD4 cell count and WHO clinical stage) retain the highest risk of TB during HAART. This may reflect the fact that the greater the degree of pre-HAART immunodeficiency, the more prolonged the period of treatment required to restore immune function. Moreover, advanced pretreatment immunodeficiency also limits the extent to which immune function can be restored in the long term [19,25,26,29]. Treatment criteria in some national antiretroviral programmes in sub-Saharan Africa, including South Africa, include only those with stage 4 disease and CD4 cell counts < 200 cells/μl (WHO 2002 recommendations [30]). Treatment according to these recommendations may limit the extent to which long-term TB-specific immune responses may be restored in many patients. This, in turn, may restrict the potential benefit of widespread use of HAART on TB control.

Our data show that TB developed among individuals who were responding to HAART. However, a more important finding was that median increases in blood CD4 cell count were much smaller among those who developed TB than in those who did not. Although virological response rates were also lower among those who developed TB, the difference was not statistically different and did not provide an explanation for the major difference in CD4 cell responses. Acute TB itself causes a transient CD4 lymphocytopenia, which may in part reverse during treatment [31]; however, this also would not explain the major persistent CD4 cell count difference between the two groups. The likeliest explanation is that a proportion of HIV-infected patients, especially those with low nadir CD4 cell counts, have poor CD4 cell responses to HAART [32,33] that may in some be related to lack of viral suppression. These patients are likely to retain a chronically heightened risk of TB.

Despite the major beneficial effects of HAART, TB incidence rates in this cohort after 5 years of HAART were still approximately 1000 cases per 100 000 population every year. Further longer-term studies of larger cohorts are needed to determine whether incidence rates continue to decrease beyond 5 years of HAART. Adjunctive strategies to reduce the risk of TB further should also be explored. Such interventions might include ones that boost immunological recovery during HAART, vaccination during HAART to increase TB-specific immunity or use of isoniazid prophylaxis coadministered with HAART. Such use of isoniazid within the South African national antiretroviral programme would raise two important issues. First, stavudine is used within the first-line regimen and concurrent use of isoniazid is likely to increase the high rates of drug-induced neuropathy [34]. Second, incidence rates of active TB during the initial months of HAART in local community-based programmes are extremely high [19], giving rise to the possibility that significant numbers of patients with undiagnosed active TB might inadvertently receive isoniazid monotherapy. Consequently, the practicalities and the risk–benefits of such a strategy would have to be carefully evaluated.

In summary, long-term HAART confers a greater reduction in TB risk than previously reported and HAART may, therefore, contribute more to TB control in low-income countries than previously estimated. TB risk is not only strongly associated with advanced baseline immunodeficiency but also with suboptimal CD4 cell count responses during HAART. Additional strategies to

reduce TB rates further during HAART need to be explored.

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ISSUES IN MEDICINE

Tuberculosis control in South Africa – will HAART help?

Stephen D Lawn, Robin Wood

The countries of southern Africa are currently at the epicentre of the HIV pandemic, and as a consequence of this annual tuberculosis (TB) notification rates in these countries have increased 2- to 5-fold since 1990.[1] An estimated 2.4 million new TB cases and 540 000 TB-related deaths occur in sub-Saharan Africa annually and this regional epidemic is fuelling a global increase in TB incidence of 1% per annum.[1,2] In response to this HIV-associated TB epidemic the World Health Organization (WHO) Committee for Africa declared an African regional emergency mandating 'urgent and extraordinary actions'.[3]

In South Africa some communities have among the highest TB incidence rates in the world, with annual TB notification rates in some townships in the Western Cape exceeding 1 000/100 000 in 2003.[4] We have more recently described a community with TB notification rates approaching 1 500/100 000 and rates of over 2 500/100 000 among those aged 30 - 49 years.[5] The majority of this disease burden is HIV-associated, and even in townships where HIV prevalence rates are now stabilising, the sobering reality is that TB incidence rates are likely to continue to rise in these communities for a further number of years.[2,5] This lag between the epidemics is because as the HIV epidemic in a community matures, the proportion of individuals with advanced immunodeficiency continues to increase after HIV prevalence has plateaued. Thus, although TB incidence has reached unprecedented levels in many communities heavily affected by HIV, further increases may occur for some years to come in the absence of further effective interventions.

Although the WHO TB control strategy based on DOTS (directly observed treatment, short course) is central to TB control efforts globally, it has failed to contain the African TB epidemic. It is now clear that although the DOTS strategy is necessary, it is insufficient. Additional measures are needed in countries with high HIV prevalence. The WHO has therefore formulated a strategic framework aimed at functional integration of control programmes for TB and HIV/AIDS.[6] It is hoped that a combination of multiple interventions may provide a more concerted approach to these epidemics.

Highly active antiretroviral treatment (HAART) potentially has a significant role to play within that strategic framework. Since HAART is associated with huge reductions in HIV-associated morbidity and mortality,[7,8] one might anticipate that, as access to HAART is scaled up in resource-limited settings, this might have a beneficial impact on TB control. In support of this supposition, numbers of cohort studies from both high-income and resource-limited settings have demonstrated that HAART reduces TB incidence by 70 - 90% during short-term follow-up.[9] In a study in Cape Town this benefit was observed among patients with a broad range of baseline blood CD4 cell counts and WHO clinical stages of disease.[10] Paradoxically, however, mathematical modelling calculations suggest that widespread use of HAART may have very limited impact on TB burden.[11] Here we explore the reasons why.

The proven efficacy of HAART in reducing short-term risk of TB in a treatment cohort does not necessarily reflect the likely long-term impact of HAART as a TB control intervention at the community level. Four additional factors are also of great importance.

Long-term risk of incident TB during HAART

Antiretroviral treatment (ART) is extremely effective in reducing the risk of end stage opportunistic infections such as cryptococcal meningitis and pneumocystis pneumonia by increasing CD4 cell counts above a safe threshold level. In contrast, the risk of TB is increased even among patients with high CD4 cell counts and so a safe CD4 cell count threshold for minimising risk of TB does not exist. Moreover, although HAART substantially restores functional TB-specific immune responses, this is almost certainly incomplete in the long term.[9] This is likely to explain why TB incidence rates in the Cape Town AIDS cohort have remained elevated at 1 000/100 000/year after 5 years of HAART.[12] Furthermore, in a community-based treatment cohort in Gugulethu, we estimate that the TB incidence of individuals receiving HAART for 3 years

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remains more than 5-fold greater than the rate among non-HIV-infected individuals living in the same community (S Lawn – unpublished data, 2006). Thus, although HAART is associated with a very substantial reduction in risk of TB, the effect is nevertheless suboptimal.

Effective ART coverage

The effective coverage of HAART (reflecting both access and adherence to treatment) achieved in a community is obviously another important factor affecting the impact of HAART on TB control. Although high levels of adherence have been demonstrated to be achievable in South African communities,[13] national HAART coverage was estimated at just 10 - 14% in June 2005.[14] Therefore, at present the low levels of coverage are unlikely to have much impact on TB rates at the national level.

How early HAART is initiated

In high TB prevalence settings, the timing of HAART initiation may have a major impact on an individual's lifetime risk of TB. If HAART is initiated late in the course of the disease, a significant proportion of HIV-infected individuals will have had TB before initiating HAART. For example, in a community-based ART service in Gugulethu, patients have a median baseline CD4 cell count of < 100 cells/µl;[15] 51% of them have had TB before programme entry, most of which was HIV-associated (S Lawn – unpublished data, 2006). HAART would have to be commenced earlier in the course of disease to have a more significant impact on TB burden in these individuals.

In South Africa utilisation of the WHO 2002 recommendations[16] in the national antiretroviral treatment guidelines[17] restricts treatment in the public sector to those with an AIDS diagnosis or a CD4 cell count < 200 cells/µl. In contrast, many other countries in sub-Saharan Africa are using the revised 2003 guidelines,[18] which recommend earlier initiation of therapy. One of the consequences of South African policy may be to limit the potential impact of ART on TB-related morbidity and mortality as is suggested by previous mathematical modelling calculations.[11]

Increased life expectancy

A further critical variable in determining the impact of HAART on TB control is the extent to which this treatment prolongs life. We have shown that HAART in Cape Town leads to a dramatic decrease in HIV-associated mortality.[14] If patients do not die then they of course remain at risk of developing TB and adding to the community burden of disease. Therefore, if HAART were to reduce the annual risk of TB 10-fold, for example, and yet prolong life expectancy 10-fold, then there would be no net reduction in the individual's lifetime risk of TB during HAART. If risk of TB were reduced 10-fold and life expectancy increased 15-fold, however, then lifetime risk of TB could paradoxically increase.

In conclusion, HIV is driving the current TB epidemic in South Africa and rates of HIV-associated TB may continue to increase even in communities where HIV prevalence has stabilised. HAART roll-out is unlikely to have a substantial beneficial impact on this TB epidemic in the foreseeable future because: (*i*) although TB risk reduction is substantial during HAART it is incomplete; (*ii*) patients typically initiate HAART with advanced immunodeficiency and this is associated with high risk of TB both before and during early HAART; (*iii*) community coverage with HAART is currently low; and (*iv*) life expectancy is increased by HAART, greatly extending the period during which patients may develop TB. Sentinel communities with good disease surveillance are needed to better understand the dynamics of the TB epidemic during HAART roll-out. Meanwhile, increased resources are needed to strengthen the South African HIV prevention and TB control programmes. Additional TB control strategies such as active TB case finding and use of isoniazid prophylaxis need to be evaluated and where appropriate implemented urgently.

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Impact of Antituberculosis Treatment on Virological Response to Highly Active Antiretroviral Therapy: Implications for Resource-Limited Settings?

To the Editor—Breen et al. [1] reported a retrospective analysis of virological responses to highly active antiretroviral treatment (HAART) among patients in London who were receiving concomitant treatment for tuberculosis (TB). Despite the potential for pharmacokinetic drug interactions and the increased risk of treatment interruptions due to drug intolerance or cotoxicity, virological responses in these patients were nevertheless similar to those observed in control subjects who did not have TB. This is an important finding. The authors reasoned that "specialist care provided by a team experienced in treating both tuberculosis and HIV could overcome the potential difficulties" (p. 1437) in the concurrent management of these 2 infections. Moreover, the fact that the group of patients with TB received 5 different drug regimens that contained 31 different drug combinations was perceived by the authors as reflecting a need for highly individualized care, which was potentially responsible for the positive outcomes. If highly individualized care delivered by specialists is required, then this has great implications for the provision of care to the huge number of patients with TB receiving HAART in resource-limited settings.

A majority of the global burden of HIV-associated TB is in sub-Saharan Africa [2], and rates in southern Africa have reached almost unprecedented levels [3]. Access to antiretroviral treatment in the region is expanding, but it can only be delivered using a simplified public-health approach, rather than individualized patient care. At a community-based public-sector antiretroviral treatment program in Cape Town, South Africa, which now treats >2000 patients, 25% of patients at entry to the program are already receiving anti-TB treatment or have active TB [4]. This burden of TB presents a huge challenge for the delivery of clinical care and has the potential to undermine program outcomes. However, similar to the findings of Breen et al., we recently reported that concurrent anti-TB treatment does not affect virological or immunological responses [4]. HIV loads were suppressed to <400 copies/mL in >94% of patients at 16 and 48 weeks whether the patients received concurrent treatment containing rifampicin for TB or not. HAART was delivered using a simplified public-health approach, in accordance with World Health Organization guidelines [5], with a nonnucleoside reverse-transcriptase inhibitor (NNRTI)–based first-line regimen. Thus, excellent virological outcomes among antiretroviral-naive patients receiving anti-TB treatment can be achieved on a large scale without specialist care and using a single standard HAART regimen.

The coadministration of rifampicin is known to reduce plasma concentrations of NNRTIs [6], which has led to debate over the optimal doses of NNRTIs for patients receiving rifampicin. Breen et al. did not report the doses used in patients who received NNRTI-based regimens in their study [1]. Studies in Thailand, however, have shown that standard doses of efavirenz and nevirapine achieve adequate plasma concentrations in most patients receiving rifampicin [7, 8]—when such patients were randomized to receive either 600 or 800 mg of efavirenz daily, equivalent virological responses were observed at 48 weeks [9]. Our data from South Africa agree with these findings; excellent virological responses were achieved when a standard daily dose of 600 mg of efavirenz was used in patients receiving treatment for TB [4].

A further critical issue for clinicians treating patients with HIV-associated TB is the decision of when to commence HAART during anti-TB treatment. Although early initiation might reduce risk of HIV-associated morbidity or mortality, this may increase the risks of immune reconstitution disease, cotoxicity, and pharmacokinetic interactions [6, 10]. However, the data of Breen et al. and those from our study in Cape Town indicate that no improvement in immunological or virological outcomes is derived from delaying HAART. These data therefore add weight to the argument favoring the early initiation of HAART.

In summary, excellent virological responses to HAART can be achieved among patients receiving concurrent anti-TB treatment using a standard dose efavirenz-based regimen delivered by a simplified public-health approach in resource-limited settings, without specialized care. The fact that virological responses are not undermined by the early initiation of HAART during anti-TB treatment supports policies favoring the early initiation of HAART.

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Reply to Lawn and Wood

To the Editor—We thank Lawn and Wood for their interest in our data and for the valuable information they provide regarding the successful management of tuberculosis (TB) and HIV coinfection in an antiretroviral program in Cape Town. Despite the 31 different drug combinations used in our cohort, 62% of individuals who received highly active antiretroviral therapy (HAART) concurrently with anti-TB therapy were prescribed a regimen that contained a single nonnucleoside reverse-transcriptase inhibitor (NNRTI), as is most widely available in resource-poor settings [1]. The large number of drug combinations that we used partly reflects the fact that, during the time period studied, the number of available antiretroviral agents and their suggested effective combinations changed. In addition, 26% of our patients were receiving HAART at the time when they received their diagnosis of TB, and a significant number had previously received antiretrovirals; it is these individuals in particular whom we suggest benefited from specialized care.

The dosing of NNRTIs administered concomitantly with rifampicin is undoubtedly an important area that has yet to be fully elucidated. In our cohort, we followed national guidelines based on published pharmacological data that suggested using efavirenz 800 mg daily in individuals weighing >50 kg and 600 mg daily in individuals weighing <50 kg [2, 3]. This produced excellent outcomes with no observed increase in rates of adverse events, compared with those in patients receiving other HAART regimens [4]. For reasons of practical ease and cost, it would be beneficial to be able to use a 600-mg dose in all cases, but this requires further study in all ethnic groups.

We agree that the timing of HAART initiation remains controversial and that data that inform this decision are welcome. The data reported from our cohort and that of Lawn and Wood certainly provide no reason to delay HAART, and we observed no increase in treatment-limiting adverse events according to whether HAART was commenced within 2 months of the TB diagnosis or later [4]. The risk of paradoxical reactions does, however, appear to be increased if HAART is started early [5, 6]. We believe that the effect of those variables that appear to be associated with clinical outcome should be assessed in a well-planned, prospective study.

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The Vaginal Flora of Healthy Women Is Not Always Dominated by *Lactobacillus* Species

To the Editor—Marrazzo's interesting editorial commentary [1] about the "enigmatic ecological mystery" of bacterial vaginosis (BV) fails to take into account the

recent findings of several investigations [2–5] that call into question some long-held assumptions about this disorder. Her essay begins with the pronouncement that we have known for decades that the normal vagina is dominated by hydrogen peroxide–producing lactobacilli and then follows this with the declaration that decreases in the numbers of lactobacilli have been associated with a host of problems, including BV, gonorrhea, HIV infection, and cervicitis. However, several investigations conducted using cultivation-independent methods have shown that a significant proportion (~30%) of healthy women lack appreciable numbers of *Lactobacillus* species.

A critical problem in studies of the etiology of BV is the diagnostic criteria used. Although numerous studies have shown that women with high numbers of *Lactobacillus* species do not have BV, it is a logical fallacy to conclude that women whose vaginal communities have few or no *Lactobacillus* species have BV. Formally, this is termed a "fallacy of propositional logic" (it is also known as "denial of the antecedent"). Unfortunately, this fallacy is the premise of the Nugent criteria [6] and is a component of the Amsel criteria [7], which are widely used for the diagnosis of BV—for these criteria, the degree of "healthiness" is assessed by scoring the abundance of *Lactobacillus* species by microscopic analysis of a Gram-stained smear or wet mount prepared from a vaginal sample.

We postulate that, because of this logical fallacy, BV is often misdiagnosed. This could partly account for the reported high incidence of so-called asymptomatic BV in reproductive-age women [8] and could also explain a proportion of BV treatment failures and apparent recurrences of BV in women. Acknowledgment that not all vaginal communities of healthy women are dominated by *Lactobacillus* species would also be in accordance with the observation that the vaginal communities of postmenopausal women (not receiving hormone-replacement therapy) often lack *Lactobacillus* species, yet these individuals do not exhibit other untoward symptoms.

We suspect that the causes of and cures for BV will continue to be enigmatic until it is recognized that, although "normal and healthy" can be equated with high numbers of lactobacilli, the converse—"unhealthy" being equated with low numbers of or no lactobacilli—is not necessarily true. We must be vigilant and recognize that, for a significant proportion of women, normal and healthy can also occur in the absence of appreciable numbers of *Lactobacillus* species.

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Reply to Forney et al.

To the Editor—I could not agree more with Forney et al.'s reminder [1] that our understanding of what constitutes "normal" vaginal flora is incomplete and that the criterion standard for diagnosing bacterial vaginosis (BV)—the Nugent criteria [2]—may become tarnished as molecular methodologies redefine the ecosystem of the vaginal environment. In fact, my intention—perhaps an overly subtle one—in putting quotation marks around the term "normal" in the first paragraph of my editorial was to question what we currently believe contributes most to vaginal health. Although the focus of my editorial was on the natural history of BV as reported by Bradshaw et al. [3], whose analyses did not involve molecular analyses of subjects' vaginal flora, it is useful to remind the *Journal*'s readers of the recent expansion of literature in this important area, which has been extensively reviewed elsewhere by Fredricks and myself [4]. As these studies progress, it will be critical to (1) determine whether the presence of vaginal bacteria (including previously undefined bacteria in the *Clostridiales* order, *Atopobium vaginae, Eggerthella* species, and *Megasphaera* species) that are detected more easily or solely by noncultivation techniques is associated with women's own perceptions of abnormal vaginal symptoms and with examination findings that are suggestive of a disrupted vaginal environment; (2) perform the same analyses with respect to detection of individual vaginal *Lactobacillus* species; and (3) carefully assess the relationship between molecular profiles of vaginal bacteria and concurrently collected vaginal Gram stains as well

as between these profiles and individual components of the Amsel criteria [5].

Jeanne Marrazzo

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Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa

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Objective: To determine the short-term and long-term risks of tuberculosis (TB) associated with CD4 cell recovery during antiretroviral therapy (ART).

Design: Observational community-based ART cohort in South Africa.

Methods: TB incidence was determined among patients ($n = 1480$) receiving ART for up to 4.5 years in a South African community-based service. Updated CD4 cell counts were measured 4-monthly. Person-time accrued within a range of CD4 cell count strata (CD4 cell strata) was calculated and used to derive CD4 cell-stratified TB rates. Factors associated with incident TB were identified using Poisson regression models.

Results: Two hundred and three cases of TB were diagnosed during 2785 person-years of observation (overall incidence, 7.3 cases/100 person-years). During person-time accrued within CD4 cell strata 0–100, 101–200, 201–300, 301–400, 401–500 and more than 500 cells/μl unadjusted TB incidence rates were 16.8, 9.3, 5.5, 4.6, 4.2 and 1.5 cases/100 person-years, respectively ($P < 0.001$). During early ART (first 4 months), adjusted TB rates among those with CD4 cell counts 0–200 cells/μl were 1.7-fold higher than during long-term ART ($P = 0.026$). Updated CD4 cell counts were the only patient characteristic independently associated with long-term TB risk.

Conclusion: Updated CD4 cell counts were the dominant predictor of TB risk during ART in this low-resource setting. Among those with baseline CD4 cell counts less than 200 cells/μl, the excess adjusted risk of TB during early ART was consistent with 'unmasking' of disease missed at baseline screening. TB incidence rates at CD4 cell counts of 200–500 cells/μl remained high and adjunctive interventions are required. TB prevention would be improved by ART policies that minimized the time patients spend with CD4 cell counts below a threshold of 500 cells/μl.

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Keywords: Africa, antiretroviral, CD4 cell, HIV, immune reconstitution, resource-limited country, tuberculosis

Introduction

In recent years, access to antiretroviral therapy (ART) has been rapidly scaled up in the countries of sub-Saharan Africa, which have borne the brunt of the dual tuberculosis (TB) and HIV epidemics. ART is associated with a 70–90% decrease in TB incidence rates among treated individuals [1–6] and is therefore a potentially important intervention

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to address the HIV-associated TB epidemic. Despite this, however, studies from within the region have reported that rates of TB during ART persist at levels much higher than background rates [7–11].

This high burden of TB is a key challenge to ART services in sub-Saharan Africa as this is a major cause of morbidity and mortality and concurrent administration of TB treatment and ART is problematic [9,11–13]. These congregate clinical settings are also associated with substantial risk of transmission of both drug-susceptible and multidrug-resistant TB [14,15]. Furthermore, high persisting rates of TB during treatment may substantially undermine the potential for ART to effect TB control at the population level [9,16,17].

Potential means to reduce this burden of TB might include initiation of ART at higher CD4 cell counts [17] and use of the interventions included within the World Health Organization (WHO) '3Is policy' [18]. However, these approaches must be based on a clear understanding of clinical epidemiology of TB during ART. How TB risk changes over time in association with immune recovery in the short-term and long-term has not been clearly defined. Although some reports suggest that TB rates are particularly high during the initial weeks of ART due to immune-mediated 'unmasking' of subclinical disease [19,20], this phenomenon has not previously been quantified. Of particular importance, the CD4 cell count threshold above which TB rates are minimized during ART-induced immune recovery is not known. To address these questions, we analysed data collected prospectively over 4.5 years of follow-up of a large ART cohort in Cape Town, South Africa.

Methods

Study population

The ART service in Gugulethu township in Cape Town has been described previously [21,22]. The district has a predominantly African population of over 300 000, the vast majority of whom live in conditions of low socioeconomic status. At the time of the study, the antenatal HIV seroprevalence was approximately 30% and the annual TB notification rate exceeded 1500/100 000. National ART guidelines were based on WHO 2002 recommendations [23], providing free treatment for those with a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count less than 200 cells/μl. First-line ART consisted of stavudine, lamivudine and a non-nucleoside reverse transcriptase inhibitor (predominantly efavirenz). Treatment compliance was high with over 90% of patients achieving viral load suppression less than 400 copies/ml [22] and with a virological failure rate of approximately 2% of patients per year [24]. In keeping with current national practice, patients receiving ART did not receive isoniazid preventive therapy (IPT).

A TB screening questionnaire was used routinely at baseline to identify symptomatic patients for TB investigations, and during ART investigations for TB were conducted when clinically indicated. Available tests include sputum smear fluorescence microscopy, automated liquid culture of sputum (MGIT 960; Becton Dickinson, Sparks, Maryland, USA), sputum induction, chest radiology, abdominal ultrasonography and fine needle lymph node aspiration for cytology. Microbiological specimens were processed within nationally accredited laboratories.

Blood CD4 cell counts (CD4 cell counts) and plasma viral load measurements were done routinely at baseline and 4-monthly during ART together with clinical review. Patients had open access to the clinic in the event of intercurrent illnesses. Detailed structured clinical and laboratory records were maintained for every patient visit. Data were transferred on a weekly basis to an electronic database. Patients requiring hospital admission were referred to a nearby 200-bed facility. Information on in-patient care was gained from discharge letters, hospital and laboratory records and post-mortem examinations. Deaths and losses to follow-up were ascertained by active community-based follow-up as previously described [25].

Collection of data on this study population for research purposes was approved by the Research Ethics Committee of the University of Cape Town and all patients enrolled gave written informed consent.

Definitions

Incident TB was defined as the first new clinical episode of TB diagnosed during ART for which the date of onset of overt symptoms occurred after ART initiation; TB episodes were dated according to symptom onset. The terms 'early' and 'short-term' ART were used interchangeably and were defined as the first 4 months of treatment. 'Long-term' ART was defined as treatment beyond 4 months. The terms 'updated CD4 cell count' and 'updated viral load' were used to refer to follow-up measurements during ART. TB diagnoses fulfilled WHO criteria for smear-positive pulmonary TB, smear-negative pulmonary TB or extrapulmonary TB [26].

Data analysis

Data were analysed using STATA version 10.0 (College Station, Texas, USA). Data from all patients who initiated ART between September 2002 and March 2006 were included. Person-time at risk of TB was accrued from the date of starting ART until either occurrence of incident TB, death, loss to follow-up, transfer to another ART programme or censoring of observation in early 2007. All person-time of observation accrued during treatment of prevalent TB present at baseline and during treatment of incident TB during ART was excluded.

As CD4 cell count and viral load measurements were routinely made every 4 months, person-time was subdivided into 4-month intervals for analysis. Each interval was defined by the CD4 cell count measurement at the start of the interval; in the event of missing CD4 cell count values (<5% of all intervals), we used the mean of the two values immediately before and after the start of the interval. These intervals were categorized into CD4 cell count strata (CD4 cell strata) 0–100, 101–200, 201–300, 301–400, 401–500 and more than 500 cells/μl. Total person-time accrued within each of the CD4 cell count strata during follow-up of the cohort was calculated.

TB incidence rates in the overall cohort and within CD4 cell count strata were calculated and Kaplan–Meier (product limit) calculations were used to estimate TB-free survival. We compared TB incidence during early (0–4 months) and long-term (>4 months) ART and the incidence of TB within CD4 cell count strata using Poisson regression; results are presented as incidence rate ratios (IRRs) with 95% confidence intervals (CIs). In these analyses, variances were adjusted for clustering of person-time on individual patients using the Huber–White sandwich (robust) estimator.

In other analyses, Fisher's exact and Wilcoxon rank-sum tests were used to compare proportions and medians, respectively, and all statistical tests are two-sided at α value of 0.05.

Results

Cohort characteristics and follow-up

During the study period, 2000 consecutive patients were enrolled in the programme. Those aged less than 15 years ($n = 161$) and those who were non-naive to ART ($n = 85$) were excluded. At data censorship, 274 (15.6%) patients had not received ART because they had died ($n = 91$, 5.2%), were alive and awaiting treatment ($n = 3$, 0.2%) or had been deferred from the programme for a variety of reasons ($n = 180$, 10.3%). ART was received by 1480 (84.4%) patients; these typically had advanced immunodeficiency and many had previous TB diagnoses (Table 1).

Of those who received ART, 155 (10.5%) died during treatment, 165 (11.2%) were lost to follow-up and 91 (6.1%) were transferred or moved out of area. The remaining 1069 (72.2%) patients were alive and receiving ART at the time data were censored. Patients were followed up for a median of 2.1 years [interquartile range (IQR), 1.5–2.7; range, 1.0–4.5 years]. Overall 2785 person-years of observation accrued during follow-up with person-time during TB treatment excluded.

During ART, 203 patients developed TB and they had broadly similar baseline characteristics as patients who did not develop TB except that those developing incident TB were less likely to have prevalent TB at baseline (Table 1). In those in whom disease site was specified ($n = 198$), 147 (74%) had pulmonary and 51 (26%) had extrapulmonary disease. Overall 64% of cases were microbiologically confirmed. Kaplan–Meier estimates of TB-free survival proportions at 1, 2 and 3 years were 0.89, 0.85 and 0.82, respectively.

Association between tuberculosis incidence rates and CD4 cell counts

The overall TB incidence rate during follow-up was 7.28 cases/100 person-years (95%CI, 6.32–8.36). Rates in the 1st, 2nd, 3rd, 4th and 5th years of the cohort were 12.5

Table 1. Baseline characteristics of patients who initiated antiretroviral therapy and of those who did or did not develop tuberculosis during treatment.

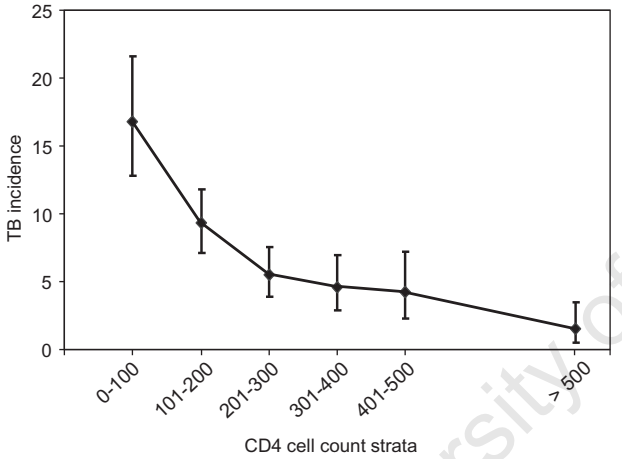
	Total ($n = 1480$)	Incident TB ($n = 203$)	No incident TB ($n = 1277$)
Mean age (years)	34.0	33.2	34.1
Men	448 (30)	56 (28)	392 (31)
Baseline WHO stage 1 and 2	294 (20)	35 (17)	259 (20)
3	825 (56)	112 (55)	713 (56)
4	360 (24)	56 (28)	304 (24)
Baseline CD4 cell count			
Median (IQR)	97 (47–155)	95 (46–143)	97 (47–155)
≥150	388 (27)	45 (23)	343 (28)
100–149	308 (22)	50 (25)	258 (21)
50–99	351 (25)	49 (25)	302 (25)
<50	375 (26)	54 (27)	321 (26)
Baseline viral load			
Median (IQR)	4.84 (4.44–5.25)	4.86 (4.50–5.26)	4.85 (4.44–5.26)
≥5.0	570 (41)	92 (44)	478 (40)
<5.0	829 (58)	118 (56)	711 (60)
History of previous TB	686 (46)	116 (57)	570 (45)
TB treatment at baseline	448 (30)	32 (16)	416 (33)

Values represent numbers (%) unless otherwise stated. CD4 cell counts in cells/μl. Viral load in $\log_{10}$ copies/ml. IQR, interquartile range; TB, tuberculosis.

(10.6–14.7), 4.5 (3.2–6.1), 3.2 (1.8–5.5), 4.5 (1.8–9.2) and 2.2 (0.1–12.5) cases/100 person-years, respectively.

To provide greater insight into changes in TB incidence rates and the association with underlying immune recovery, we next calculated TB rates stratified according to updated CD4 cell counts categorized into 0–100, 101–200, 201–300, 301–400, 401–500 and more than 500 cells/μl CD4 cell count strata. A strong graded association was observed with the highest TB rates during person-time accrued within the less than 100 cells/μl CD4 cell stratum and the lowest during person-time accumulated within the more than 500 cells/μl CD4 cell stratum (Fig. 1).

Poisson regression models were used to examine risk factors for incident TB during long-term ART. A very strong independent association between TB risk and updated CD4 cell counts during ART was observed (Table 2). The adjusted TB rate associated with the lowest CD4 cell stratum was more than nine-fold higher than the rate associated with the highest CD4 cell stratum. TB risk was not, however, independently associated with baseline patient characteristics, updated viral load measurements or duration of ART, which was included to control for any survival effect not reflected by updated CD4 cell counts.



CD4 count stratum	No. of incident TB cases	Person-years of observation (PYO)	Rate per 100 PYO (95%CI)
≤ 100	59	352.0	16.76 (12.76-21.62)
101-200	65	701.2	9.27 (7.15-11.82)
201-300	38	693.7	5.48 (3.88-7.52)
301-400	23	499.1	4.61 (2.92-6.91)
401-500	13	307.1	4.23 (2.25-7.24)
> 500	5	334.2	1.50 (0.49-3.49)

Fig. 1. Graph of tuberculosis incidence rates (95% confidence interval, cases/100 person-years) plotted against serially updated CD4 cell counts measured during total duration (early and late) of antiretroviral therapy. CD4 cell counts (cells/μl) were measured at baseline and 4-monthly during antiretroviral therapy (ART). Tuberculosis (TB) incidence rates are seen to decrease with increasing CD4 cell counts. Data used to derive these rates are displayed in the table beneath. Median CD4 cell counts within the 0–100, 101–200, 201–300, 301–400, 401–500 and more than 500 CD4 cell strata were 60, 157, 250, 345, 446 and 700 cells/μl, respectively. CI, confidence interval.

Excess tuberculosis rates during early antiretroviral therapy

Thus far we have demonstrated that TB rates during ART were very strongly associated with updated CD4 cell counts. However, further analyses exploring the exceptionally high TB rates (18.8 cases/100 person-years; 95%CI, 15.2–23.3) during the first 4 months of ART found that the rate of TB during this period was much higher than that during long-term ART having adjusted for relevant covariates.

Initial unadjusted analyses showed that within the 0–100 and 101–200 cells/μl CD4 cell strata, TB rates during early ART were approximately double the rates during long-term ART (Fig. 2). In contrast, within the 201–300 cells/μl CD4 cell stratum, rates in two periods did not significantly differ. This excess TB incidence rate observed during early ART among those with CD4 cell counts 0–200 cells/μl was found to be confined to the first 4 months of treatment. After adjustment for covariates, including viral load, absolute CD4 cell count values, clustering and variable interval duration, the incidence rate during early ART remained significantly higher (adjusted IRR = 1.66; 95%CI, 1.06–2.59; $P = 0.026$). Thus, the excess proportion of TB cases presenting during early ART was 40% (95%CI, 6–61%).

High persisting tuberculosis rates during long-term antiretroviral therapy

As the extent of CD4 cell count recovery was the dominant association with long-term TB incidence rates, we next examined how CD4 cell counts changed over 4 years of ART (Fig. 3). The proportion of patients with a CD4 cell count less than 200 cells/μl decreased steeply from 89% at baseline, reaching less than 10% of patients beyond 128 weeks. Conversely, the proportion of patients attaining a CD4 cell count more than 500 cells/μl steadily increased, representing 48% of patients after 4 years (Fig. 3).

We next reasoned that the overall rate of TB in the cohort would be related to the proportions of person-time accrued at different CD4 cell count levels. Despite excellent CD4 cell count recovery resulting in a steadily increasing proportion of patients achieving a CD4 cell count more than 500 cells/μl, 88% of person-time was nevertheless associated with CD4 cell strata below 500 cells/μl. Thus, in this analysis, only 12% of

Table 2. Risk factors for incident tuberculosis during long-term antiretroviral therapy, excluding the first 4 months of treatment.

Patient characteristics		Crude association			Multivariate model		
		IRR	95%CI	*P*	IRR	95%CI	*P*
Age		0.99	0.97–1.02	0.605	0.99	0.97–1.01	0.307
Sex		1.04	0.73–1.48	0.845	0.99	0.67–1.46	0.969
Previous TB diagnosis		1.23	0.87–1.75	0.240	1.29	0.85–1.96	0.239
Baseline WHO stage	1 and 2	1			1		
	3	1.06	0.68–1.64	0.800	1.07	0.63–1.83	0.797
	4	1.19	0.73–1.92	0.488	1.21	0.66–2.21	0.533
Baseline CD4 cell count (cells/μl)	>150	1			1		
	101–150	1.18	0.76–1.84	0.466	1.12	0.70–1.79	0.632
	51–100	1.05	0.67–1.65	0.832	0.78	0.45–1.35	0.369
	0–50	0.94	0.59–1.50	0.795	0.66	0.37–1.18	0.162
Baseline viral load (log copies/ml)		0.88	0.71–1.10	0.258	0.92	0.71–1.20	0.548
Cohort enrolment year	1	1			1		
	2	0.81	0.53–1.26	0.353	0.67	0.43–1.04	0.078
	3	0.89	0.61–1.30	0.545	0.63	0.41–0.96	0.030
	4	0.65	0.31–1.37	0.255	0.44	0.20–0.97	0.042
Duration of ART	5–12 months	1.0			1.0		
	13–24 months	0.52	0.35–0.76	0.001	0.66	0.42–1.03	0.069
	>24 months	0.43	0.27–0.67	<0.001	0.62	0.34–1.12	0.116
Updated CD4 cell count (cells/μl)	>500	1			1		
	401–500	2.82	1.01–7.90	0.048	3.59	1.17–11.03	0.025
	301–400	3.08	1.18–8.09	0.022	3.78	1.30–11.01	0.015
	201–300	3.65	1.43–9.30	0.007	4.13	1.45–11.81	0.008
	101–200	4.86	1.93–12.26	0.001	5.42	1.79–16.47	0.003
	0–100	7.39	2.74–19.92	<0.001	9.21	2.69–31.52	<0.001
Updated viral load (copies/ml)	<400	1					
	>400	1.86	1.33–2.61	<0.001	1.29	0.88–1.87	0.192

Age and baseline viral load included as continuous variables. For baseline viral load, the IRR for a 1.0 log decrease in viral load is shown. 'Previous TB diagnosis' includes all TB diagnoses established at any time prior to ART initiation. 'Updated' CD4 cell count and viral load values are the serial measurements made 4-monthly during follow-up on ART. ART, antiretroviral therapy; CI, confidence interval; IRR, incidence rate ratio; TB, tuberculosis.

person-time was associated with the lowest TB rates achievable during ART (1.5 cases/100 person-years).

Impact of baseline CD4 cell counts on person-time within low CD4 cell strata

We also hypothesized that patients with the lowest baseline CD4 cell counts would accrue substantially more person-time within the lowest CD4 cell strata and thereby remain at high risk of TB for long periods. Indeed, during long-term ART, those with baseline CD4 cell counts of 100 cells/μl or less accrued 40% of person-time with CD4 cell counts in the range 0–200 cells/μl compared with just 17% of person-time accrued by those whose baseline counts were more than 100 cells/μl ($P < 0.001$). Thus, patients with the lowest baseline counts remained at high risk of TB for a longer period of time.

Discussion

In this analysis, we calculated TB incidence rates stratified by serially updated CD4 cell counts during ART and compared TB rates during early and long-term treatment. Several key findings emerged. Among patients with CD4 cell counts less than 200 cells/μl, there was a 1.7-fold excess adjusted TB rate during early ART compared with rates during long-term treatment ($P = 0.026$). During long-term ART, a very strong independent association between TB rates and updated CD4 cell counts was observed. At CD4 cell counts of 200–500 cells/μl, TB incidence rates remained high but were significantly lower at CD4 cell counts exceeding a threshold of 500 cells/μl. However, despite excellent immune recovery, patients spent a large majority of time at CD4 cell counts less than 500 cells/μl and overall TB rates in the cohort were, therefore, high. These data substantially extend the findings of previous studies [7–11], providing important insights that will assist in the development of approaches to address the challenge of HIV-associated TB.

We suggest that the excess TB rates during early ART among those with CD4 cell counts less than 200 cells/μl may be due to ART-induced 'unmasking' of subclinical TB that was present but unrecognized at baseline [20,27]. In patients who develop 'unmasking' TB, rapid immune recovery is thought to trigger host inflammatory responses and development of symptomatic disease [20]. Although some overt cases of 'unmasking TB' have

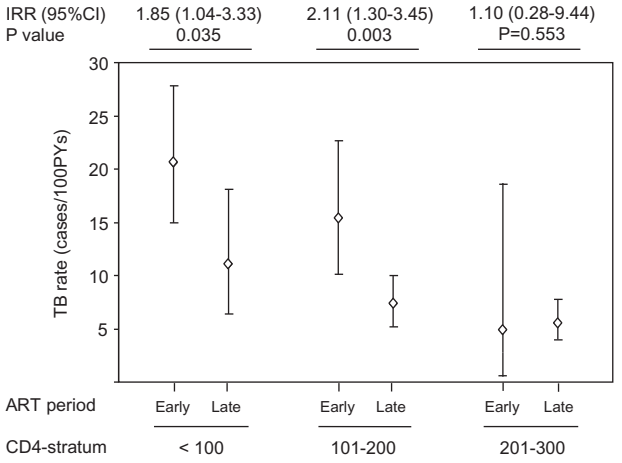


Fig. 2. CD4 cell-stratified tuberculosis incidence rates during first 4 months of antiretroviral therapy (early antiretroviral therapy) and during person-time thereafter (late antiretroviral therapy). Within each of the CD4 cell strata 0–100, 101–200 and 201–300 cells/μl, tuberculosis (TB) incidence rates during early antiretroviral therapy (ART) are compared with rates during late ART [cases/100 person-years, 95% confidence interval (CI)]. Incidence rates and incidence rate ratios (IRRs) for these CD4 cell strata are shown. Within the two lowest strata (0–100 and 101–200 cells/μl), TB incidence rates during early ART were approximately double the rates during long-term ART in unadjusted analyses and 1.7-fold higher in adjusted analyses ($P = 0.026$).

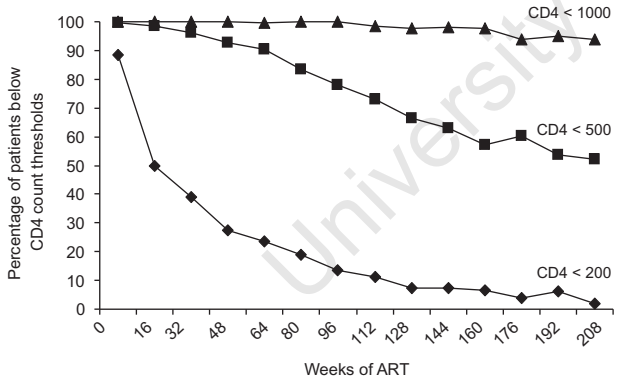


Fig. 3. Changes in CD4 cell counts during 4 years of antiretroviral therapy. The graph shows the changes in the proportions (%) of patients with CD4 cell counts lying below thresholds of 200, 500 and 1000 cells/μl with increasing duration of antiretroviral therapy (ART). During the first year of ART, the proportion of patients with a CD4 cell count less than 200 cells/μl decreased steeply from 89% at baseline, eventually accounting for less than 10% of patients. In contrast, the proportion of patients with CD4 cell counts more than 500 cells/μl increased steadily over time reaching 48% after 4 years. Only data from patients with at least two serial measurements were included and the numbers of patients represented at 0, 48, 96, 144 and 208 weeks were 1313, 1129, 595, 237 and 97, respectively.

been described [19,20,28–31], this phenomenon lacks a clear clinical case definition [27] and has not previously been quantified.

Several lines of indirect evidence support our hypothesis. First, high rates of subclinical, culture-proven TB have been detected in patients enrolling for ART in this [32] and in other HIV cohorts in Africa [33–36]. Immune recovery in the first 4 months of ART in this cohort is very rapid, even in those with low baseline CD4 cell counts [37]. In keeping with 'unmasking' TB, excess rates were restricted to those with baseline CD4 cell counts less than 200 cells/μl and were confined to early ART [19,20,27,28]. Further corroboration is derived from a study of Ugandan children in whom a more than two-fold increase in TB rates during the initial months of ART was attributed to 'unmasking' TB [38].

These data suggest that 'unmasking' TB may account for over one-third of TB cases presenting during the initial months of ART in this setting. CIs around this estimate are wide, however, and confirmatory studies are required. Pre-ART investigations for TB were routinely done only in those with suggestive symptoms or clinical signs. These data suggest the potential need for routine microbiological screening for TB at baseline in all patients starting ART in this setting and this approach is supported by the findings of a more recent study in this cohort [32].

Although we have previously found that CD4 cell counts were strongly associated with TB incidence rates during ART [9], the present study used a novel analytic approach to derive CD4 cell-stratified TB rates, yielding important new insights. A steep risk gradient was observed between the highest and lowest CD4 cell strata with a more than 9-fold difference in adjusted rates. We have used a similar analytic approach to examine changing mortality risk in this cohort [39]. Whereas mortality risk was found to be largely minimized by the attainment of an updated CD4 cell count of more than 200 cells/μl, the present study shows that a threshold of more than 500 cells/μl has to be exceeded to minimize TB rates. Thus, eligibility criteria for ART initiation that aim to minimize mortality risk are not optimal for TB prevention.

Immune recovery in this cohort compared very favourably with that observed in ART cohorts in high-income countries [40]. Approximately half of the patients achieved a CD4 cell count more than 500 cells/μl after 4 years of ART (Fig. 3) and in these patients, TB rates (1.5 cases/100 person-years) remained approximately only two-fold higher than the rate among HIV-seronegative adults in a comparable neighbouring community (0.7 cases/100 person-years) [41]. However, despite excellent immune recovery, the large majority of person-time in this cohort accrued at CD4 cell counts less than 500 cells/μl, with TB rates ranging between 4.2 and 16.8 cases/100 person-years.

As a result, the overall TB incidence rate in the cohort was high (7.3 cases/100 person-years), approximately 10-fold higher than the rate in HIV-seronegative adults in these communities [41].

Although this analysis only examined incident TB from the time of ART initiation, other factors occurring just prior to this may have influenced the findings. Many patients enrolling in this cohort had recently completed TB treatment, potentially conferring a relative protection against further TB episodes [9]. Although all person-time accrued during TB treatment was excluded from the analysis, a similar protective effect may also have been present during the period following TB treatment in the many patients with TB diagnoses at baseline. These effects may have reduced the unadjusted TB incidence rates among those with the lowest baseline CD4 cell counts.

In multivariate analysis, baseline CD4 cell counts did not have any predictive value for TB risk over and above that provided by the current CD4 cell count at any given time-point. This does not support the hypothesis that lower baseline CD4 cell counts are associated with increased risk of clinically significant persisting defects in TB-specific immune function during long-term ART [17]. Importantly, however, patients with low baseline CD4 cell counts accrued much greater person-time within low CD4 cell strata, thereby remaining at high TB risk for longer periods. Thus, whereas current CD4 cell counts are the key predictor of instantaneous TB risk, baseline CD4 cell counts are key predictors of cumulative long-term risk of TB during ART as was similarly found for mortality [39].

Data from this study provide insight into the strategies needed to reduce the long-term burden of incident TB. Most fundamentally, the time that patients spend at low CD4 cell counts less than 500 cells/μl needs to be minimized. This requires both earlier HIV diagnosis and initiation of ART at higher CD4 cell counts. Unfortunately, the current South African national ART policy restricts eligibility to those with AIDS or a CD4 cell count of less than 200 cells/μl and therefore greatly undermines the potential benefits of ART for TB prevention. A change in this policy is needed to reduce both high mortality rates [39] and to improve TB control.

Adjunctive TB prevention strategies such as the WHO '3Is policy' [18] are also needed to reduce TB in ART services. Within this policy, intensified case finding (ICF) might be done not only at baseline but also serially (e.g. 6-monthly) during at least the first year of ART when TB rates are highest. This approach might particularly target those with persistently low CD4 cell counts. Use of IPT concurrently with ART is likely to reduce long-term TB rates [42] but data from randomized controlled trials are awaited [43]. However, initiation of IPT at the same time as ART may be problematic because high rates of subclinical active TB at baseline and 'unmasking' TB during the first 4 months of ART may inadvertently lead to many patients with active TB receiving isoniazid monotherapy. In light of our findings, a logical approach might be to consider initiating IPT after completion of the first few months of ART.

Strengths of this study include good patient retention and ascertainment of outcomes, frequent monitoring of CD4 cell counts and the novel analytic approach. Some person-time may have been misclassified as CD4 cell counts continuously change over time. Some TB disease may have remained unascertained among those who died, leading to underestimation of TB rates particularly in those with the lowest CD4 cell counts. Not all TB cases were proven by culture of *Mycobacterium tuberculosis,* although the rates and proportions of pulmonary and extrapulmonary disease reported are entirely consistent with other data from this setting [6,8,9,41]. The multiple lines of evidence for 'unmasking' TB during early ART are indirect and yet provide a coherent and biologically plausible explanation.

Baseline characteristics of the patients were typical of patients in ART roll-out programmes across Africa, but rates of loss to follow-up were comparatively low [44]. Such losses are not related to degree of immunodeficiency in this cohort [25] and so we do not suspect they affected the TB rates observed. The countries of southern Africa are the areas of the world hit hardest by the TB and HIV epidemics and the absolute TB rates recorded are likely to be higher than those in other regions. Nevertheless, the key relationship between TB risk and updated CD4 cell counts is likely to be applicable in other settings.

In conclusion, low baseline CD4 cell counts and 'unmasking' of subclinical TB are likely to explain the high burden of TB during the first 4 months of ART. This may potentially be reduced by initiation of ART at higher baseline CD4 cell counts and more effective screening for TB at baseline. The high long-term TB incidence is strongly associated with the proportion of person-time at CD4 cell counts less than 500 cells/μl and adjunctive TB prevention interventions are undoubtedly needed. However, the impact of ART on TB prevention in low-resource settings would be greatly improved by ART policies that minimize the time patients spend with CD4 cell counts less than 500 cells/μl.

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Tuberculosis-associated immune reconstitution disease: incidence, risk factors and impact in an antiretroviral treatment service in South Africa

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Objective: To determine the burden and impact of immune reconstitution disease (IRD) associated with tuberculosis (TB) among patients initiating antiretroviral treatment (ART) in sub-Saharan Africa.

Design: Retrospective analysis of a study cohort enrolled over 3 years within a community-based ART service in South Africa.

Methods: Patients receiving treatment for TB at the time ART was initiated (n = 160) were studied. Cases of TB-associated IRD during the first 4 months of ART were ascertained.

Results: The median baseline CD4 cell count was 68 cells/μl [interquartile range (IQR), 29–133 cells/μl) and ART was initiated after a median of 105 days (IQR, 61–164 days) from TB diagnosis. Although IRD was diagnosed in just 12% (n = 19) of patients overall, IRD developed in 32% (n = 12) of those who started ART within 2 months of TB diagnosis. Pulmonary involvement was observed in 84% (n = 16) and intra-abdominal manifestations were also common (37%). Overall, 4% (n = 7) of the cohort required secondary level health-care for IRD and two (1%) patients died. In multivariate analysis, risk of IRD was strongly associated with early ART initiation and low baseline CD4 cell count. Of patients with CD4 counts < 50 cells/μl, the proportions who developed IRD following initiation of ART within 0–30, 31–60, 61–90, 91–120 and > 120 days of TB diagnosis were 100%, 33%, 14%, 7% and 0%, respectively.

Conclusions: The risk of TB-associated IRD in this setting is very high for those with low baseline CD4 cell counts initiating ART early in the course of antituberculosis treatment. However, most cases were self-limiting; overall secondary health-care utilization and mortality risk from IRD were low.

AIDS 2007, **21**:335–341

Keywords: immune reconstitution disease, antiretroviral treatment, tuberculosis, resource-limited country, Africa

Introduction

A majority of the global burden of HIV-associated tuberculosis (TB) lies in sub-Saharan Africa [1] and rates in southern Africa have reached almost unprecedented levels [2]. Antiretroviral treatment (ART) is now becoming more widely available in the region. However, the fact that many patients accessing ART are already receiving treatment for TB [3,4] presents a major clinical challenge due to the complexities involved in

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the concurrent management of these two infections [5–8]. In addition to high pill burden, drug co-toxicity and pharmacokinetic drug interactions, TB-associated immune reconstitution disease (IRD) has been reported in up to 43% of patients in high-income countries receiving concurrent treatment for these infections [9,10].

Since the prevalence of TB among patients entering ART programmes in sub-Saharan Africa is extremely high [3,4], TB-IRD could greatly complicate the delivery of ART in the region, causing substantial in-programme morbidity and mortality and increasing the burden on secondary health-care facilities. Moreover, the risks associated with TB-IRD are important variables in the debate over the optimal time for ART initiation in patients with TB [7,8,10]. There are, however, no existing published reports of the frequency and impact of TB-IRD in ART programmes in sub-Saharan Africa.

Data concerning the burden of TB-IRD in ART programmes in sub-Saharan Africa are clearly needed to inform both clinicians and those involved in the development of treatment guidelines for ART programmes. We have previously described a community-based ART programme in Cape Town, reporting detailed analyses of morbidity, mortality, immunological recovery and overall programme outcomes [3,11–15]. Within this service 25% of patients have prevalent TB at programme entry, the majority of whom subsequently receive overlapping antituberculosis treatment and ART [3]. Collection of detailed prospective data on this large cohort over 3 years provided the opportunity to analyse the burden of TB-IRD in this setting.

Methods

The ART service based at the Gugulethu Community Health Centre in Cape Town was started in 2002 and has previously been described in detail [11–13]. The district has a predominantly African population of over 300 000, the vast majority of whom live in conditions of low socioeconomic status. In 2003 the antenatal HIV seroprevalence was 28% and the TB notification rate exceeded 1 000/100 000 [16]. Patients were referred from primary care HIV clinics to the ART programme. National guidelines for use of ART were based on the WHO 2002 recommendations [17], which advise treatment for those with a prior AIDS diagnosis (WHO stage 4 disease) or a blood CD4 cell count < 200 cells/μl. First-line ART comprised stavudine, lamivudine plus a non-nucleoside reverse transcriptase inhibitor (predominantly efavirenz). Treatment compliance rates were very high as reflected by rates of viral load suppression < 400 copies/ml, which exceeded 90% at all follow-up time-points over the first 3 years of the programme [11]. In addition to the minimum schedule of clinic appointments at 4, 8, and 16 weeks, patients with clinical problems such as concurrent TB were reviewed more frequently. All patients had open access to the clinic for medical problems during weekdays and 24-hour access to care by the nearby secondary hospital. All care and medication was supplied free of charge to the patients.

The high burden and diagnostic criteria for TB in this programme have been described in detail [3]. Available investigations for TB included sputum smear microscopy and culture (MGIT, Becton Dickinson, Sparks, Maryland, USA), chest radiology, abdominal ultrasonography, fine needle aspiration of lymphadenopathy for cytology and culture and drug sensitivity testing of mycobacterial isolates. Nebulized sputum induction was accessible when required. Results of investigations for TB were cross-checked with electronic records held by the National Health Laboratory Service.

Detailed structured clinical and laboratory records were maintained for every patient visit. Data were transferred on a weekly basis to an electronic database. Patients requiring in-patient care were referred to a nearby 200-bed secondary hospital. Information on in-patient care was gained from discharge letters, hospital and laboratory records and post-mortem examinations. Deaths and losses to follow-up were ascertained by active community-based follow-up as described in detail previously [12,13]. Outcomes were determined for all patients. Attributable causes of death were assigned based on all the available information after detailed review by two specialists in infectious diseases and HIV medicine.

Definitions

Patients with 'prevalent TB' included those receiving antituberculosis treatment at entry to the ART programme as well as patients with new TB diagnoses established after enrolment in the programme but prior to ART initiation. TB-IRD was diagnosed among patients with prevalent TB who had an initial symptomatic improvement during antituberculosis treatment followed by deterioration of symptoms after initiation of ART, which was not due to another opportunistic infection, drug adverse effect, ineffective TB treatment or drug-resistant TB [10].

Data analysis

All patients enrolled into the programme between September 2002 and April 2005 were studied and the period between ART initiation and the 4-month follow-up appointment was studied. In analyses, Fisher's exact and Wilcoxon rank-sum tests were used to compare proportions and medians, respectively. Multiple logistic regression was used to examine the associations between IRD and patient demographic and clinical characteristics. Regression diagnostics followed standard procedures [18]. All P-values reported were two-sided at alpha $= 0.05$.

Results

Patients and follow-up

A total of 756 ART-naive patients who enrolled during the study period initiated ART. Their median age was 33 years [interquartile range (IQR), 28–37 years] and 73% were female. Antituberculosis treatment was being received at the time of ART initiation by 160 (21%) patients. Those with prevalent TB differed from those who were TB-free in having a lower median CD4 cell count (68 cells/μl; IQR, 29–133 cells/μl) versus 101 cells/μl (IQR, 52–156 cells/μl; $P < 0.001$), a higher median viral load (4.96 versus 4.84 log RNA copies/ml; $P = 0.003$) and more advanced WHO stages of disease (stages 3 and 4 among 62% and 38% versus 51% and 26%, respectively). Since many patients had initiated antituberculosis treatment prior to entering the programme whereas others had TB diagnosed after enrolment, the duration of the interval between TB diagnosis and ART initiation was very broad (median 105 days; IQR, 61–164 days).

Of the patients with TB (n = 160), 142 (89%) were retained on ART at the 4-month follow-up appointment. Of the remaining patients, one (0.6%) was transferred-out at week 14, one (0.6%) was lost to follow-up at week 5, and 16 (10%) died. Overall a total of 60.1 person-years of observation accrued during follow-up. Deaths were due to advanced Kaposi's sarcoma (n = 4), acute sepsis (n = 3), cryptococcal immune reconstitution disease (n = 2), renal failure (n = 1), and wasting syndrome with profuse chronic diarrhoea (n = 2). Two patients died of TB without evidence of IRD: one had very advanced hypoxic lung disease and the other had active disease due to TB treatment default. Two patients died with TB-IRD as described below.

Cases of TB immune reconstitution disease

Among patients with prevalent TB, a total of 19 (12%) were diagnosed as having TB-IRD. Symptoms developed a median of 2 weeks (IQR, 1.5–3.5 weeks) after initiation of ART and systemic as well as organ-specific symptoms were present in all. IRD presented as an exacerbation of existing disease manifestations alone in 10 patients with pulmonary disease among the majority (n = 9) (Table 1). Seven further patients with initial diagnoses of pulmonary TB developed new disease manifestations at another anatomic site as well as a concurrent exacerbation of respiratory disease in five (Table 1). Two other patients with disseminated disease developed IRD that culminated in death; both had pulmonary, intra-abdominal and bone marrow involvement. These two cases occurred early in the history of the ART programme; since diagnoses were not established ante-mortem, neither was managed as IRD.

Overall, intra-abdominal manifestations occurred in 7 (37%) IRD cases. Hepatomegally with elevated serum concentrations of alkaline phosphatase and gamma-glutamyl transferase but relatively normal serum concentrations of alanine transaminase were detected in four patients (21%). IRD was self-limiting in the majority of patients. Secondary level out-patient or in-patient care was received by three and four patients, respectively, two received oral corticosteroids and one required a laparotomy.

Table 1. Clinical manifestations of tuberculosis (TB)-associated immune reconstitution disease (n = 19).

Exacerbation of existing disease manifestations (n=10)
deterioration of pulmonary TB (n = 9)
deterioration of cervical lymphadenopathy (n = 1)
Development of new disease manifestations at another anatomic site with or without exacerbation of existing disease (n = 7)
cervical lymphadenopathy (n = 1)
hepatomegally (n = 2)
intra-abdominal lymphadenopathy (n = 1)
tuberculous terminal ileitis with perforation and peritonitis (n = 1)
cervical and intra-abdominal lymphadenopathy, hepatomegally and splenic micro-abscesses (n = 1)
tuberculous arthropathy of left hallux (n = 1)
IRD resulting in death (n = 2)
death due to deterioration of disseminated TB with pulmonary, intra-abdominal and bone marrow involvement

Risk factors for TB immune reconstitution disease

Comparison of the characteristics of TB patients who developed IRD (cases) with those who did not is shown in Table 2. Cases had significantly lower baseline CD4 cell counts and ART was initiated after a shorter interval from

Table 2. Characteristics of patients with tuberculosis (TB) (n = 160) who did or did not develop TB-associated immune reconstitution disease (TB-IRD).

Patient characteristics	TB-IRD cases (n = 19)	IRD-free patients (n = 141)	*P*
Baseline characteristics			
Median age (years)	35	32	0.60
Female [n (%)]	11 (58)	105 (74)	0.13
Median CD4 count (cells/μl)	31	74	0.003
Patients in CD4 cell categories (cells/μl) [n (%)]			
>150	0	26 (18)	
100–149	2 (11)	28 (20)	
50–99	5 (26)	42 (30)	
<50	12 (63)	45 (32)	0.03
Median viral load (log copies/ml)	5.13	4.96	0.27
Extrapulmonary TB (%)	4 (21)	40 (28)	0.59
Time from TB diagnosis to ART (days)			
Median	40	117	<0.001
Patients in time categories [n (%)]			
>120	1 (5)	64 (45)	
91–120	2 (11)	28 (20)	
61–90	4 (21)	23 (16)	
31–60	5 (26)	20 (14)	
0–30	7 (37)	6 (4)	
Follow-up at 4 months			
Median change in CD4 count (cells/μl)	83	88	0.97
Proportion with VL<400 copies/ml (%)	100	96	0.47

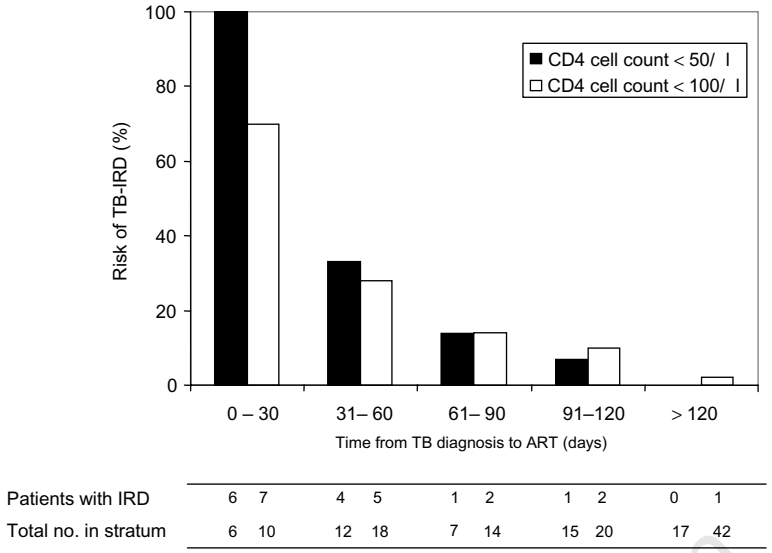

Fig. 1. Graph showing the risk of TB-IRD among patients stratified by baseline CD4 cell count and by the interval between TB diagnosis and initiation of ART (days). The proportion (%) of patients in each stratum who developed TB-IRD is displayed graphically. The numbers of cases of TB-IRD together with the total number of patients in each stratum is displayed beneath the graph.

TB diagnosis. There was no significant correlation between the baseline CD4 cell count and the delay between TB diagnosis and ART (*r*, 0.09; $P = 0.259$). Cases did not differ from those who remained IRD-free with respect to other baseline characteristics or laboratory results at 4 months' follow-up (Table 2).

Among patients with baseline CD4 cell counts < 50, 50–99, 100–149 and ≥ 150 cells/μl, IRD developed among 21%, 14%, 7% and 0%, respectively. Although only 12% of patients overall developed TB-IRD, the proportion of patients affected who commenced ART within 2 months of TB diagnosis was much higher (32%). Furthermore, the risk of IRD for patients with low baseline CD4 cell counts commencing ART early in the course of antituberculosis treatment was especially high (Fig. 1).

Multivariate analysis that included all baseline characteristics showed that increased odds of developing IRD was significantly associated with lower baseline CD4 cell counts but that the association with shorter delays from TB diagnosis to ART initiation was particularly strong (Table 3).

Table 3. Multivariate analysis for risk factors for TB-associated immune reconstitution disease (TB-IRD) among patients with prevalent TB receiving antiretroviral treatment (ART) (n = 160).

	RR of IRD	95% CI	*P*
Age[1]	1.03	0.93–1.13	0.596
Sex			
Female	1.00		
Male	3.07	0.74–12.74	0.123
Baseline CD4 count[a,b]	0.983	0.970–0.997	0.018
Baseline viral load[a]	2.39	0.68–8.34	0.172
TB site			
Pulmonary	1.00		
Extrapulmonary	0.59	0.12–3.00	0.525
TB diagnosis to ART delay (days)			
> 90	1.00		
61–90	6.64	1.20–36.5	0.030
31–60	10.6	1.88–59.5	0.007
0–30	69.5	9.94–485.6	< 0.001

[a]Analysed as continuous variables and thus the relative risk (RR) reflects the change in RR of TB-IRD associated with a 1-unit increase in the independent variable.
[b]A decrease in CD4 cell count of 100 cells/μl is associated with a 5.3-fold increase in the relative risk of developing IRD ($P = 0.020$). CI, Confidence interval; RR, relative risk.

Discussion

The proportion of patients with HIV-associated TB reported to develop IRD following initiation of ART in high-income countries ranges between 11% and 43% [9,19–22]. This wide variation is likely to reflect differences in cohort characteristics, case definitions and differences in the mean time interval between TB diagnosis and ART initiation. Data from resource-limited countries on TB-IRD is scarce; a rate of 8% was reported from India [23] but a study from Tanzania reported no cases at all [24]. This study from South Africa is the largest series patients with overlapping ART and antituberculosis treatment yet reported. We found that overall 12% of TB patients developed IRD. However, the low rate is likely to reflect the fact that many patients were referred to the ART programme having already completed several months of antituberculosis treatment (median, 3.5 months) and so many were likely to be at low risk of

IRD. Risk of TB-IRD was much higher among those with early initiation of ART, affecting 32% of patients initiating ART within 2 months of TB diagnosis. The rate among these patients, a more relevant comparison group, is consistent with rates reported from high-income countries [9,20–22].

Most previous reports of TB-IRD are from series of patients attending referral hospitals [9,20,21,23]. In contrast, this is the first report of TB-IRD from a community-based ART programme in a resource-limited setting. An important strength of this analysis is that all patients within the programme receiving TB treatment at the time of ART initiation were included and outcomes for all patients are known except for one who was lost to follow-up. Data collection was detailed and prospective; access to unscheduled clinic visits when needed was good, and all treatment was free of charge. As a result, data completeness is likely to be high. All patients were ART-naive and received a standard first-line ART regimen, excluding these as potential variables affecting risk of IRD. In common with most previous studies [9,19,20,22,23], diagnoses of IRD were made retrospectively but were nevertheless based on systematically collected prospective data. Although some cases of TB-IRD have been reported to occur >16 weeks of ART [10], only one case occurred beyond 12 weeks and so we restricted this analysis to the first 16 weeks of ART. The retrospective study design may have missed some very mild manifestations of TB-IRD but, because of excellent cohort retention and close follow-up of cases, all clinically relevant disease is likely to have been detected and thus the impact of TB-IRD on this ART programme should have been accurately assessed. The focus of this study was on IRD presenting as clinical deterioration of prevalent TB rather than 'unmasking' of new TB presenting after ART initiation.

As the overall size of the cohort of TB patients was large, we were able to perform multivariate analysis to identify risk factors associated with development of IRD. As reported previously, early initiation of ART was associated with a higher risk of IRD [10,20,22]. However, in view of the broad range of the intervals between TB diagnosis and ART initiation, we were able to define this relationship more clearly than has been done previously (Fig. 1, Table 3). Risk of IRD was significantly increased if ART was commenced within 3 months from TB diagnosis but increased greatly when ART was initiated within 1 month. However, as the numbers of patients and cases within each time stratum are small, these data should not be taken as precise estimates of risk of TB-IRD within each stratum.

In addition to the timing of ART, we also found that increased risk of IRD was also significantly associated with lower baseline CD4 cell counts (Tables 2 and 3). A similar trend has been observed in some [19,21] but not other [9,20,23] studies. Compared to other studies, a higher mean baseline CD4 count may explain the unusually low rate of IRD in a study from India [23]. The association of low CD4 cell count with increased risk of IRD is plausible as those with the most advanced HIV-associated immunodeficiency are likely to have higher mycobacterial antigen load and greater impairment of immune responses that may rapidly reverse during early ART [25]. Some previous studies have also found extrapulmonary TB to be associated with increased risk of IRD [9,19,20]. However, in resource-limited settings, patients with a diagnosis of pulmonary TB are often not investigated further for evidence of asymptomatic extrapulmonary involvement, probably resulting in substantial under-diagnosis in those with advanced immunodeficiency.

Existing studies do not show consensus on whether early immunovirological responses to ART are risk factors for IRD [9,20–22] although the largest of these studies did find that risk was increased among those with a more rapid fall in viral load [22]. In this cohort, rates of virological suppression and immunological recovery were exceptionally high but were not discriminatory as risk factors for IRD. However, these measurements were made at 16 weeks of ART and not at the onset of IRD. In view of the timing of these measurements, however, these data strongly suggest that following development of IRD, treatment compliance and responses to ART thereafter were not undermined.

Manifestations of TB-IRD are diverse, but literature from high-income countries [10] and from India [23] reported cervical lymphadenitis much more frequently than was observed in this study. This is likely to reflect true differences in the spectrum of disease since cervical adenitis is clinically readily apparent and is therefore unlikely to have been missed. Respiratory manifestations were most common but a high rate of intra-abdominal disease was also observed, including frequent hepatic involvement with elevation of serum concentrations of bile cannalicular enzymes. Many patients initially treated for pulmonary TB developed additional manifestations of IRD at extrapulmonary sites, again reflecting likely initial under-diagnosis of extrapulmonary involvement in patients with pulmonary TB. In this study, two (1%) TB patients died of IRD; both diagnoses were retrospective and neither was managed with either use of corticosteroids or discontinuation of ART. This is an important finding as a review of previous cases from high-income countries revealed that although life-threatening manifestations were reported there were no deaths [10]. This illustrates the importance of establishing clinical diagnoses of TB-IRD and the need for access to secondary level health care for some patients in this setting. However, overall mortality associated with TB-IRD in this cohort was low and the majority of TB-IRD was self-limiting. Indeed, we have previously

reported that cryptococcal IRD is a far more common cause of death in this service [12,15].

IRD could potentially lead to a high rate of secondary health-care utilization. However, just 4% of TB patients were referred for either in-patient or out-patient secondary care for this reason, representing approximately 1% of all patients in the cohort. This represents a very small proportion of total secondary health-care utilization by individuals accessing ART in this service, which was received by approximately 16% of patients during the first 16 weeks of ART (Guy Harling, unpublished data).

The factors affecting the optimal timing for initiation of ART in patients with HIV-associated TB are multiple. It has recently been shown that active TB and antituberculosis treatment during ART do not compromise immunological and virological responses to treatment in this [3] and other cohorts [26,27], suggesting that pharmacokinetic and compliance issues are not strong arguments for delaying treatment [28]. Risk of IRD has also been raised as a factor favouring delayed treatment initiation [6,8]. However, the present study suggests that the overall mortality risk associated with TB-IRD is small whereas we have previously reported that even short delays in initiation of ART are associated with a high mortality risk in this cohort [12,13]. Taken together, these data would favour a policy of earlier initiation of ART among TB patients. Our data, however, indicate that such a policy would substantially increase the proportion of patients at risk of IRD. Diagnostic and management strategies for TB-IRD in resource-limited settings must be developed, including guidelines for use of corticosteroids. Moreover, the long delays for many TB patients in this study to initiate ART are indicative of the lack of integration of TB and ART services locally. These delays and the need for coordinated management of patients with TB-IRD strongly support moves to integrate such services.

In summary, the risk of TB-associated IRD in this setting is very high for those with low baseline CD4 cell counts and early initiation of ART. Although the contribution of TB-IRD to secondary health-care utilization and mortality risk in the whole cohort was small, any future policy changes recommending early initiation of ART among TB patients would be very likely to lead to an increase in this burden of disease.

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CLINICAL

HIV/TB: WHEN IS IT SAFE TO START HAART?

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South Africa has the fourth highest burden of tuberculosis (TB) worldwide after China, India and Indonesia and has the highest TB notification rate of any country. The World Health Organization (WHO) estimated that in 2006 South Africa had 303 114 incident TB cases; of these patients, 32% were tested for HIV and 53% were found to be HIV infected.[1] HIV testing of TB cases has been encouraged by the WHO and testing has resulted in identification of increasing numbers of HIV-infected individuals in the TB control programme. The success of this policy has been demonstrated in the Cape Town Gugulethu antiretroviral clinic, where referrals directly from the local TB clinics have increased from 15% to 30% within the past 2 years. The national TB control programme has therefore become an increasingly important pathway to HIV care and access to highly active antiretroviral therapy (HAART). An additional 15 - 20% of patients in the Gugulethu programme have a diagnosis of TB made during the HAART screening period, further increasing the number of individuals on TB medication who require HAART. Mortality after referral is very high. The HIV/TB case mortality has been reported to be as high as 16 - 35%[2] prior to the introduction of HAART, with both HIV and TB contributing to this mortality. Optimal timing of HAART is currently unknown and there is an urgent need for development of evidence-based protocols for HAART initiation and immune reconstitution disease (IRD) management.

Tuberculous meningitis occurring in HIV-infected individuals (HIV/TBM) exemplifies the dilemmas facing clinicians when addressing potentially preventable mortality. HIV/TBM has a devastating clinical impact with a median time from onset of symptoms to presentation of 10 days, 67% mortality and a median time to death of 20 days.[3] Expert opinion on when to start HAART in HIV-infected patients with TB meningitis varied between 2 weeks and 12 months after starting TB medications.[4] This uncertainty of expert opinion reflects the present lack of randomised clinical trial data with which to inform clinical management. A clinical trial specifically addressing immediate initiation versus deferring HAART (zidovudine/lamivudine/efavirenz) for 8 weeks has been conducted at two hospital sites in Ho Chi Minh city, Vietnam. Results of this study should become available in late 2008 or early 2009.[5] A study demonstrating proof of the concept that earlier initiation of antiretroviral therapy may impact on mortality of HIV patients with active opportunistic infections (OIs) was recently presented.[6] The AIDS Clinical Trials Group study 5164 (ACTG 5164) was a randomised strategy trial of immediate versus delayed ART in the setting of acute OI. At the time of inclusion study subjects had pneumocystis pneumonia (63%), cryptococcal meningitis (13%), other acute pneumonic illnesses (10%) or multiple opportunistic infections (30%). Patients were randomised to immediate or delayed initiation of HAART, a median of 12 days or 45 days after starting OI treatment, respectively. After 48 weeks, deaths in the early treatment group were significantly lower with no difference in drug toxicities, adherence or hospitalisation. Somewhat counter-intuitively, IRD was also less frequent in the earlier treatment group. The conclusion from this study was that in the absence of contraindications very early use of HAART should be considered in patients with acute OIs. However, it should be noted that TB cases were not included in this study population.

CONSIDERATIONS DETERMINING EARLIER VERSUS LATER INITIATION OF HAART

The decision when to initiate HAART after TB treatment is complex, involving a number of variables including treatment tolerance, drug co-toxicities, pharmacokinetic drug interactions and impact of polypharmacy on adherence (Fig. 1). However, of over-riding importance is the mortality associated with delays in ART initiation versus mortality associated with IRD when HAART is initiated early. The frequency of IRD in cohort studies describing co-infected patients varies markedly between 8% and 43%.[7,8] The mean interval to IRD after HAART initiation also varies widely (1 - 180 days) with most cases occurring within the first 28 days.[7] However, cross-cohort comparisons are complicated by differing mortality in cohorts from high- and low-resourced settings and

differing incidences of TB-associated IRD in TB patients starting HAART in different settings. Furthermore, while variable rates of IRD may represent differences between cohorts, analysis is complicated by variable ascertainment and lack of a standardised IRD definition. A consensus document with proposed definitions of IRD for use in resource-constrained settings may help address the problem of differing case definitions.[8] IRD is characterised by worsening of systemic symptoms, transient enlargement of pre-existing lesions, onset of new lesions including lymphadenopathy, and worsening of radiographic changes. Life-threatening conditions are rare but include tracheal and bronchial obstruction, pulmonary adult respiratory distress syndrome (ARDS), central nervous system tuberculomas and cerebral oedema.[7-13] The management of life-threatening IRD includes use of high-dose steroid therapy and may necessitate interruption of HAART, although there are no randomised controlled studies to inform policy. The precise pathological processes responsible for IRD are not clearly defined, but the condition is associated with an expansion of CD4 cells in the peripheral blood[8,14] and increased macrophage activity.[15] Fig. 2 illustrates a proposal that clinical manifestations result from an interplay between cellular events and mycobacterial antigen load.[16] The risk factors for development of IRD are predominantly a low CD4 cell count and a short interval between starting TB therapy and HAART initiation.[9,13,17] In a prospective Cape Town cohort, IRD occurred in 100% and 70% of patients commencing HAART within 30 days with CD4 counts of <50 cells/µl and 50 - 100 cells/µl respectively[13] (Fig. 3). Extrapulmonary TB and black ethnic group have been identified as additional risk factors for IRD.[9] Severe TB-associated IRD therefore tends to develop in those patients who have a high mortality risk, manifested by low CD4 cell counts and a high mycobacterial burden associated with disseminated TB. Several studies reporting considerable morbidity associated with IRD have not shown an excess mortality.[13,17-21] Similar findings were also reported in a South African cohort where 10.5% who developed TB-IRD died; however, 9.9% of TB patients who did not develop IRD also died.[9] Development of IRD and IRD-associated mortality in these studies was therefore not associated with significant excess overall mortality.

Variations in IRD frequency and associated mortality indicate that the optimal timing of ART initiation may differ between settings. In lower income countries, the risk of mortality associated with delays in ART initiation is likely to outweigh the excess mortality of TB-associated IRD. The optimal timing of ART initiation may therefore be earlier in the course of TB treatment for patients in resource-limited settings compared with those in high-income settings. Current guidelines for the timing of HAART in patients with TB are shown in Table I. All these guidelines reflect an increased urgency to commence HAART at lower CD4 cell counts with variable

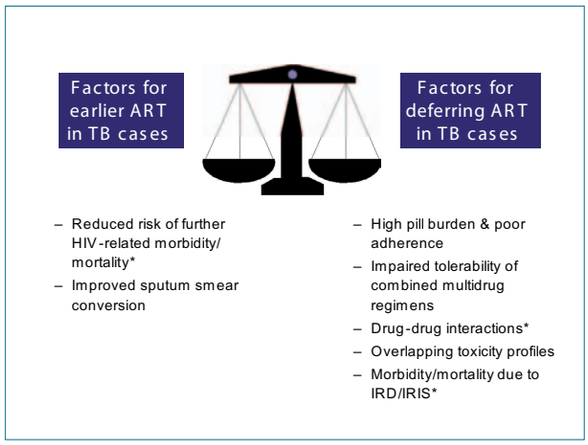

Fig. 1. Factors influencing the decision of timing of commencement of HAART after starting TB therapy in HIV-infected individuals.

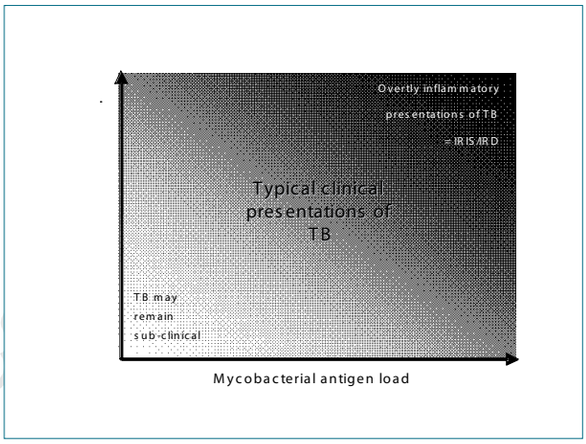

Fig. 2. The proposed interaction between Mycobacterium tuberculosis *antigen load and rate and intensity of immune recovery after initiating HAART (adapted from Lawn* et al.[16]*).*

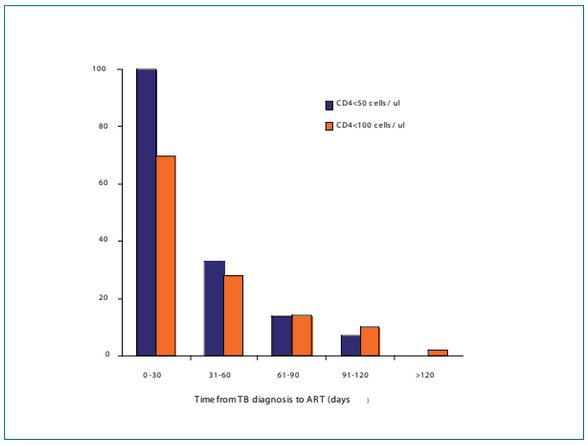

Fig. 3. The impact of baseline CD4 cell count and timing of initiation of HAART on risk of TB-immune recovery disease in the Gugulethu cohort, Cape Town (adapted from Lawn et al.[13]*).*

timing recommendations due to a lack of data from randomised controlled trials. This lack of informative data is clearly reflected in the recommendations of the International AIDS Society (IAS), USA. Several guidelines focus on 8 weeks as a key time point in TB therapy when simplification of TB medications occurs. In South Africa schedule 1 TB therapy consists of an intensive four-

TABLE I. CURRENT INTERNATIONAL AND SOUTH AFRICAN GUIDELINES FOR THE TIMING OF INITIATION OF HAART IN HIV-INFECTED PATIENTS ON TB THERAPY

Year	Organisation	CD4 count	Recommendations
2003	American Thoracic Society*	CD4 <350 CD4 >350	Individualise between 4 and 8 wks Defer HAART
2004	MMWR† (Pediatrics)		Defer 4 - 8 wks
2004	WHO 'Scaling up ART in resource-limited settings'‡	CD4 <200 CD4 200 - 350 CD4 >350	2 wks - 8 wks Start at 2mo. Defer HAART
2004	South African National ART Programme§	CD4 < 50 CD4 <200 CD4 >200	2 wks - 8 wks 8 wks 6 mo.
2006	IAS/USA panel¶		Individualise as there are no RCTs
2008	DHHS: Guidelines	CD4 <100 CD4 100 - 200 CD4 200 - 350 CD4 >350	2 wks 8 wks 8 wks 8 - 24 wks or defer

Guidelines available at: *http://www.thoracic.org; †htpp://www.cdc.gov/mmwr/; ‡http://www.doh.gov.za; §http:// www.iasusa.org/pub/; ¶http:// www.aidsinfo.nih.gov/Guidelines/; ¶http://www.who.int/3BYS/publications/documents/ARV_guidelines/en/
MMWR = ; WHO = World Health Organization; IAS = ; DHHS = .

drug therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) which is reduced to two-drug maintenance (RIF/INH) after 8 weeks.[22] The South African TB control programme promotes use of fixed-dose combination tablets which results in identical pill burdens before and after the 8-week treatment time point. Rifampicin, the anti-TB agent with the greatest potential for drug-drug interactions with non-nucleoside and protease inhibitor antiretrovirals, is continued throughout the whole 6 months of treatment. Similarly isoniazid, with a potential for peripheral neuropathy co-toxicity with stavudine, is also continued throughout TB therapy. The main co-toxicity shared between TB and HAART is hepatotoxicity, and some staggering of initiation of the two treatment regimens may simplify clinical management of drug-induced hepatitis. Although pyrazinamide, which is routinely discontinued after 8 weeks of TB treatment, may contribute somewhat to hepatic co-toxicity, it is unproven whether the optimal deferring time period is 8 weeks,

STUDY DATA ADDRESSING WHEN TO START HAART

Randomised controlled trials addressing the optimal timing of ART initiation in patients with TB are awaited, but meanwhile data from observational cohorts and modelling studies may help inform policy. Cohort studies have reported a markedly variable impact of HAART on TB mortality.[19,23-26] Three cohort studies describing outcomes in patients starting HAART at different time points after TB therapy reported at the International AIDS Society 2008 Conference meeting in August 2008 highlight the difficulties in interpreting cohort data. A Brazilian clinic-based cohort study of 662 patients found no significant difference in survival between patients starting HAART in the first 2 months, 2 - 6 months or more than 6 months after commencement of TB treatment.[24] In contrast, an Iranian study of 69 hospitalised patients showed significant increases in TB cure rate and survival in patients who started HAART within 2 weeks compared with 8 weeks of TB treatment.[25] A third study, from Argentina, showed similar differences in TB cure rate and survival with early initiation of HAART.[26] However, this last study also reported significant differences in baseline characteristics between the groups, demonstrating that cohort studies may be subject to considerable selection bias. A South African cohort study of the International Epidemiological Databases to Evaluate AIDS Group (IeDEA) retrospective analysis of 4 000 HIV/TB patients from multiple sites in the Free State and Cape Town will be completed and is planned for reporting during 2009.[27]

A decision analysis model, based on published cohort data, examined three treatment strategies in patients with AIDS and TB; early initiation of HAART (<2 months), deferred HAART (>2 months), and no HAART strategy.[28] The model indicated that earlier HAART could reduce mortality at 1 year by 30% and 80% compared with the deferred and no HAART strategies, respectively.

Several randomised controlled studies addressing the timing of HAART after starting TB treatment are currently enrolling.[5,29-32] Of these ongoing studies only

TABLE II. ONGOING TRIALS OF HAART IN HIV-INFECTED INDIVIDUALS ON TB THERAPY[5,29-32]

Trial (sponsor)	CD4 (/µl)	Time (design)	Study design	End-points
CAMELIA (NIH/ANRS)	<200	12 mo. (ROL*)	2 wks v. 8 wks after TB initiation (*N*=660, accruing results 2009/10)	Survival
ACTG 5221 (NIAID)	<200	12 mo. (ROL*)	2 wks v. 12 wks after TB initiation (*N*=200 of 800 accrued)	AIDS-free survival
SAPIT (NIAID)	>50	18 mo. (ROL*)	<2 mo. v. >2 mo. v. post 6 - 8 mo. TB Rx (*N*=645, DSMB stopped 3rd arm)	Survival AIDS
TB-HAART (WHO/TDR)	>200 <500	6 mo. (RPC†)	HAART at 2 wks v. placebo at 2 wks (*N*=1 900 accruing results 2011)	Survival, TB failure
TB meningitis (Wellcome Trust)	All	9 mo. (RPC†)	Immediate v. 8 wks ART + steroids (*N*=247, accrued results Dec 2008)	Survival

*Randomised open-label study.
†Randomised placebo-controlled study.
NIH = National Institute of Health; ANRS = Agence Nationale Recherche sur Le Sida; NIAID = National Institute of Allergy and Infectious Disease; WHO = World Health Organization; TDR = Tropical Disease Research; ROL = ; RPO = .
Author: ROL and RPO in full please

the Vietnamese TB meningitis study is expected to be able to report outcomes in the near future (Table II). The Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA), a large randomised open-labelled study conducted in five sites in Cambodia, should be the first of these studies to report outcomes, some time during 2009 or 2010.[29] Unexpectedly, preliminary data from the Starting Antiretroviral therapy in three Time Points in Tuberculosis (SAPIT) study conducted in KwaZulu-Natal became available in September 2008 owing to the data safety and monitoring board (DSMB) discontinuing the third randomisation arm of the study because of a 55% increased mortality in subjects deferring HAART for 6 - 8 months.[31]

DISCUSSION

Determination of the optimal timing of initiation of ART in patients with TB is urgently needed in South Africa, where HIV/TB is extremely common and availability of HAART is rapidly expanding. HIV/TB case fatality rates are high and the optimal deferral time will therefore be determined predominantly by mortality rather than morbidity. Unfortunately the results of several randomised controlled trials addressing when to start HAART in TB, with a primary endpoint of survival, will not be available until 2009/2010. Meanwhile, preliminary data from the SAPIT study indicate that deferring treatment for 6 months is associated with significantly increased mortality.[31] The Vietnamese study results of immediate initiation of HAART in TBM should become available in the next few months.[5] The results of this study may not necessarily be generalisable to other forms of TB; however, any significant mortality benefit of immediate initiation of HAART will bolster support for earlier treatment in other severe forms of TB.

The present status of information concerning the most important factors that may impact on the optimal timing of HAART in TB are shown in Fig. 4. Reduction of ongoing HIV-related mortality by HAART is counterbalanced by TB/IRD-associated mortality and a clinical need to stagger the initiation of both treatments for ease of clinical management of co-toxicity. Those at highest risk of HIV progression also have the highest risk of co-toxicity and IRD. The ACTG 5164 study has demonstrated improved survival with very early initiation of HAART in patients co-infected with acute OIs.[6] Treatment of TB requires prolonged treatment and is complicated by frequent occurrence of IRD. However, published reports indicate that while TB/IRD is a common cause of morbidity it is a less frequent cause of death.

Much interest has rightly focused on the optimal timing of HAART in relation to TB treatment. In low-

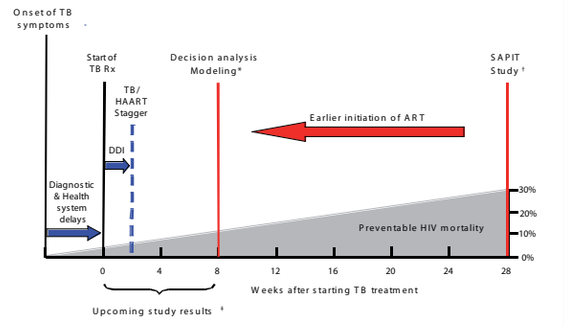

*Fig. 4. Data from present and future studies that affect the timing of initiation of HAART and are likely to impact on preventable HIV mortality in individuals already on TB treatment (*ref. 28, †ref. 31, ‡refs 29, 32).*

income settings TB in HIV-infected patients is often only diagnosed after prolonged delay, and yet the mortality associated with even short delays in accessing HAART is unacceptably high. Furthermore, the potentially more important problem of delays in the care pathway has received little attention.

While the results of randomised controlled studies addressing the timing of initiation of HAART are eagerly awaited, the results may still not be generalisable to all types of TB, HIV progression, race and health systems. In the meantime it is important to recognise that time delays between the onset of TB symptoms and starting HAART in those eligible for HAART are associated with potentially preventable HIV-related mortality and that all delays should be minimised.

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Immune Reconstitution and "Unmasking" of Tuberculosis during Antiretroviral Therapy

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Tuberculosis (TB) is the most common opportunistic disease in HIV-infected patients during the initial months of antiretroviral therapy (ART) and presents a great challenge to ART programs in resource-limited settings. The mechanisms underlying development of TB in this period are complex. Some cases may represent progression of undiagnosed subclinical disease present before starting ART, emphasizing the importance of careful screening strategies for TB. It has been suggested that progression in such cases is due to immune reconstitution disease—a phenomenon in which dysregulated restoration of pathogen-specific immune responses triggers the presentation of subclinical disease. However, whereas some cases have exaggerated or overtly inflammatory manifestations consistent with existing case definitions for IRD, many others do not. Moreover, since ART-induced immune recovery is a time-dependent process, active TB may develop as a consequence of persisting immunodeficiency. All these mechanisms are likely to be important, representing a spectrum of complex interactions between mycobacterial burden and changing host immune response. We propose that the potential range of effects of ART includes (*1*) shortening of the time for subclinical TB to become symptomatic (a phenomenon often referred to as "unmasking"), (*2*) increased rapidity of initial onset of TB symptoms, and (*3*) heightened intensity of clinical manifestations. We suggest that the term "ART-associated TB" be used to refer collectively to all cases of TB presenting during ART and that "immune reconstitution disease" be used to refer to the subset of ART-associated TB cases in which the effect on disease severity results in exaggerated and overtly inflammatory disease.

Keywords: HIV; tuberculosis; antiretroviral; immune reconstitution; IRIS

Since the mid-1990s, availability of antiretroviral therapy (ART) has transformed the prognosis of HIV-infected people living in high-income countries and more recently in resource-limited settings (1, 2). Through suppression of HIV replication, ART permits both quantitative and functional reconstitution of the immune system (3, 4), thereby reducing the risk of new opportunistic infections and neoplasia (1, 5). However, an appreciable (albeit diminished) risk of opportunistic infection persists during ART, especially during the first few months of treatment. Tuberculosis (TB) is the most frequent of these both in high-income and resource-limited settings (6–15).

Although heightened clinical vigilance may, in part, account for increased ascertainment of cases of TB during the initial months of ART, several different mechanisms may also underlie the temporal distribution of cases. ART-induced immune recovery occurs gradually over time and so active TB may develop in some patients as a consequence of persisting immunodeficiency. Conversely, restoration of pathogen-specific immune responses during treatment may cause subclinical TB to manifest or be "unmasked" (16–19). Of these latter cases, there is debate as to whether some or all of these should be termed "immune reconstitution disease" (IRD); clarity on this issue is needed.

The burden of TB during early ART is particularly great in resource-limited settings where this presents a major challenge in treatment programs (9). Management of these two diseases requires concurrent use of two multidrug regimens, with associated high pill burden, overlapping toxicity, and pharmacokinetic interactions (20). Furthermore, patients with incident TB during ART have increased mortality risk and may be a source of TB transmission within ART clinics (9, 21). Understanding the underlying mechanisms will facilitate development of preventive and therapeutic strategies. In this article, we consider how ART-induced immune recovery is likely to mediate a range of effects on both the timing and manifestations of active TB.

BURDEN OF TB DURING EARLY ANTIRETROVIRAL THERAPY

A number of reports have described the incidence of TB in patients receiving ART in high-income countries (12–15). The largest of these is a study of European and North American cohorts that included over 17,000 patients with a median baseline CD4 cell count of 280 cells/μl (12). The prevalence of TB at baseline was not known but the incidence rate was 1.3 cases per 100 person-years during the first 3 months of ART (12). Beyond this time point, the risk of TB decreased sharply (Figure 1A).

As might be expected, incidence rates of TB are substantially higher in ART cohorts in sub-Saharan Africa and other resource-limited settings (7, 9–11, 22). For example, in a community-based ART cohort in South Africa with a median baseline CD4 cell count of 96 cells/μl, prevalent TB (disease either newly diagnosed or in patients already receiving treatment) was reported among 25% of all patients at baseline (9). Despite the

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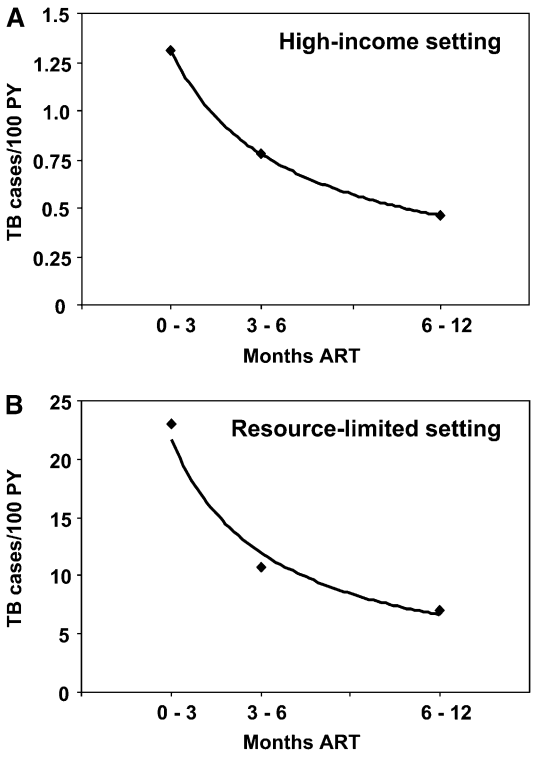

Figure 1. Graphs showing the high incidence of tuberculosis (TB) during first 3 months of antiretroviral treatment (ART) and the subsequent rapid decrease. Data are from (*A*) cohorts in low-TB-burden countries in Europe and North America (data are from Reference 12) and (*B*) a community-based ART program in Cape Town, South Africa, where the burden of TB is very high (data are from Reference 9). Power trend lines are shown. PY = person-years.

high yield of prevalent TB detected during pre-ART screening of all patients with suggestive symptoms, the rate of incident TB in the first 3 months of ART was nevertheless extremely high at 23 cases per 100 person-years (Figure 1B). All cases had new onset of symptoms after initiation of ART and therefore represented true incident disease. The risk of TB decreased steeply during the first year of ART (Figure 1B) and was strongly associated with improving immune status (9).

ROLE OF IMMUNODEFICIENCY

Patients accessing ART programs in resource-limited settings typically have advanced immunodeficiency (2) and therefore have very high TB incidence rates just before commencing ART. An exceptionally high rate in excess of 50 cases per 100 person-years has been reported in African patients with World Health Organization clinical stages 3 and 4 disease in the pre-ART era, for example (23). In cohorts in which incidence rates are so high, active but "subclinical" TB is likely to be present in a proportion of patients at any given point in time. This could either progress to symptomatic disease or remain undiagnosed until death of the patient.

Post mortem studies of patients who died with HIV/AIDS support the supposition that rates of subclinical TB are high in Africans with advanced HIV infection; occult disseminated TB has been detected in up 54% of cadavers (24, 25). Furthermore, in studies in which HIV-infected patients in southern Africa were actively screened for TB, high rates of culture-proven pulmonary disease have been detected despite the absence of suggestive symptoms (26–29). In the absence of a standard definition for subclinical TB, the proportions reported by such studies will therefore vary according to the criteria used. However, the fact that the World Health Organization's DOTS (directly observed treatment, short course) strategy places greatest emphasis on investigation of symptomatic patients is problematic and most subclinical disease is likely to remain undetected pre-ART.

The limited sensitivity of TB diagnostic tests when applied to patients with advanced immunodeficiency further compounds poor TB case-finding in patients preparing to start ART. Sputum smear microscopy, for example, which is the mainstay of TB diagnosis in much of the developing world, typically detects less than 50% of active pulmonary disease in those with HIV infection (9, 27, 30). Thus, a proportion of the TB presenting during early ART may simply reflect failure of diagnosis of suspected cases before treatment initiation. This burden will vary between settings according to the intensity of screening and diagnostic facilities available.

We suggest that subclinical TB among patients entering ART programs provides much of the reservoir of infection that fuels the high rates of symptomatic TB presenting during early ART. Because ART-induced immune recovery is time dependent, some of these cases may progress to symptomatic disease during early ART in the context of persisting immunodeficiency. In addition, rates of latent *Mycobacterium tuberculosis* infection and new exogenous infection are also very high in high-TB-prevalence settings (31, 32); these may also progress and contribute to the burden of active disease during early ART.

ROLE OF IRD

Having discussed the role of immunodeficiency, we now consider IRD as a further important potential mechanism in the presentation of TB (33–35). IRD (also known as immune reconstitution inflammatory syndrome or IRIS) is believed to be caused by dysregulated recovery of pathogen-specific immune responses during the initial months of ART. This leads to development of unusual and overtly inflammatory disease, most commonly in association with chronic viral, mycobacterial, and invasive fungal infections (33–36).

TB-associated IRD presents in two principal ways. The most common form occurs in patients with TB receiving effective treatment who subsequently start ART; ART-induced immune recovery results in the deterioration of the clinical manifestations of TB. This has been reported to occur in 8 to 43% of patients with TB starting ART (35, 37–42). In the context of this review, however, the second form of TB IRD is the more relevant. Here, recovery of host immune responses triggers the clinical presentation or "unmasking" of previously subclinical TB during the early months of ART (33–35). It seems likely that these two types of IRD may be related phenomena, but immunologic studies to support this assertion are lacking.

Reports of TB IRD causing presentation of TB after ART initiation are relatively few (16, 18, 19, 43, 44), the clearest example being that reported by Goldsack and colleagues (19). In this report, a 41-year-old African with advanced HIV in London commenced ART. Two weeks later, he presented again with rapid-onset fever, breathlessness, and hypoxia. Although his chest radiograph was normal before treatment, a repeat radiograph revealed florid miliary shadowing that was microbiologically proven to be TB. The onset of this complication was temporally associated with a rapid immunologic and virologic response to ART. The patient developed adult respiratory distress syndrome, requiring mechanical ventilatory support, but he subsequently had a good response to anti-TB drugs and adjunctive corticosteroid therapy. An appropriate diagnosis of TB

IRD was made, which is entirely consistent with similar cases we have seen in South Africa.

Breen and colleagues in London, United Kingdom, described a series of 19 cases of incident TB during ART and hypothesized that the presentation of 13 of these cases may have been attributable to IRD (16). The rationale for this was largely based on the temporal distribution of cases; 13 early-onset cases were clustered within the first 4 months of ART and many of these had a marked inflammatory response and systemic illness. Also, a paradoxical reaction after initiation of anti-TB therapy was observed in eight (62%) of the early cases compared with none of the late-onset group (16).

In agreement with the report by Breen and colleagues, workers in Uganda speculated that IRD was also the cause of the large number of new cases of TB diagnosed during ART in their setting (18). In a further report from the same country, all cases of incident TB during the first year of ART were described by the authors as being due to IRD (45). Park and coworkers reported from South Korea that 82% of new TB events occurring during the first year of ART (either new TB cases or paradoxical reactions at a new disease site) were attributable to IRD (17).

Evaluation of the role of IRD among cases of incident TB during ART is hindered by the lack of a diagnostic test or a clinical case definition specific to this condition. Importantly, however, the existing literature indicates that, for a diagnosis of IRD to be made, either the clinical manifestations and/or the clinical course of disease should be atypical and be consistent with an exaggerated, overtly inflammatory host response (34, 35, 46). Our own clinical experience gained during several studies of TB during ART in South Africa (7, 9, 42), however, is that most cases of TB diagnosed in the initial months of ART have typical clinical presentations. Only a subset of such cases present with unusual, overtly inflammatory manifestations that would be consistent with a clear diagnosis of IRD. Moreover, in high-TB-burden settings, it cannot be reasoned that the timing of onset of TB during the first months of ART is particularly unusual. Thus, in the context of the existing literature on IRD, it would appear appropriate to use the term IRD to refer to some but not all cases of incident TB during the initial months of ART.

HYPOTHESIS: A SPECTRUM OF EFFECTS OF IMMUNE RECOVERY ON TB PRESENTATION

Thus far, we have considered contrasting reasons why TB may present during early ART, with TB developing in the context of persisting immunodeficiency or TB presenting as TB IRD. However, we suggest that these represent opposing ends of a spectrum of important effects of ART-induced immune recovery on TB presentation.

Because immunopathologic host responses to *M. tuberculosis* are central to the clinical presentation of TB, we suggest that development of symptoms at a given anatomic site is a function of two key parameters: the mycobacterial antigen burden and the intensity and quality of the associated host inflammatory response. It is the interrelationship between these that is likely to determine the threshold for symptom onset and to affect the timing and severity of cases of TB presenting during early ART.

The supposition that high mycobacterial load is a risk factor for TB IRD is strongly suggested by two observations: first, the risk of IRD is greater the earlier ART is started during anti-TB therapy when the residual mycobacterial burden is high; second, the risk of TB IRD is increased in those with disseminated TB (35, 38, 42). Considerable improvements in immune function occur during the initial weeks of ART in most ART-naive patients living in resource-limited settings, even in those with the lowest baseline CD4 cell counts (47–49). Thus, the potential for cases of TB presenting in this period to be modified by changes in immune function is substantial. We suggest that immune recovery has three principal effects on the progression of subclinical TB to symptomatic disease, namely the timing of onset, the rapidity of initial symptom onset, and the overall intensity of clinical manifestations.

Timing of Onset

In an HIV-infected patient with a certain level of immune function, symptoms of TB are likely to develop at a given anatomic site once the mycobacterial burden that is sufficient to trigger host inflammatory responses has been reached (Figure 2A). If ART is initiated, however, TB-specific immune function increases rapidly (49, 50), and this would have the effect of rapidly lowering the threshold for symptom development (Figure 2B). This process may be augmented by immune-mediated liberation of mycobacterial antigen, further enhancing immune responses. In patients with subclinical TB, this would cause TB to manifest much sooner than would otherwise have occurred in the absence of ART (Figure 2B). In effect, the initiation of ART may serve as a therapeutic challenge that unmasks subclinical disease, triggering the presentation of TB. This accelerated progression

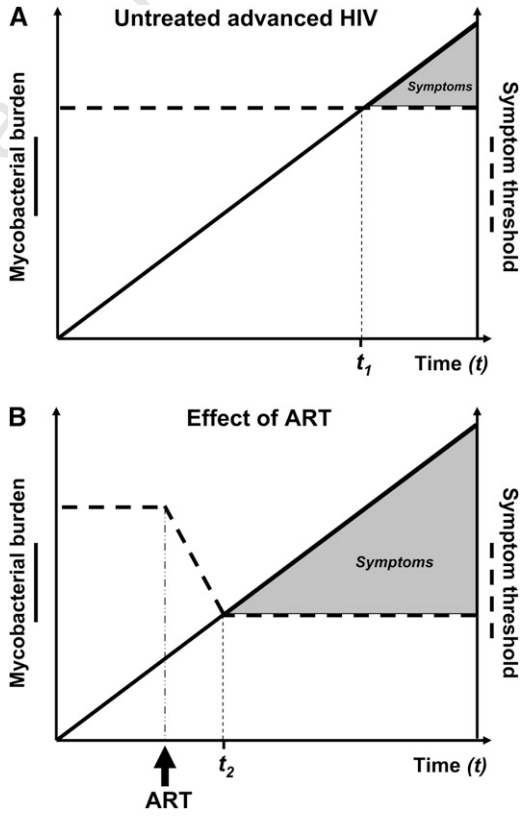

Figure 2. (*A*) A hypothetical graph showing the rising burden of *Mycobacterium tuberculosis* over time at a given anatomic site in an HIV-infected patient with advanced immunodeficiency (shown as a linear rise over time for simplicity). Symptom onset occurs when the mycobacterial load rises to a level at which the host inflammatory responses are triggered (symptom threshold). With poor immune function, the symptom threshold is reached at time t_1. (*B*) If the patient were to have started antiretroviral therapy (ART), tuberculosis-specific immune function would increase rapidly and the threshold for development of symptoms would correspondingly decrease. As a result, symptoms would develop much earlier soon after the initiation of ART (time t_2).

to symptomatic TB would result in the temporal clustering of cases in the initial 3 months of ART as observed in Figure 1 and in the report by Breen and colleagues (16).

Rapidity of Onset

In patients with advanced immunodeficiency, TB often has a fairly insidious onset. In contrast, however, our clinical impression is that, in cases of TB presenting during the initial weeks of ART, the initial onset of symptoms is often unusually rapid. This is consistent with the speed with which antimycobacterial immune responses recover during early ART (49, 50).

Intensity of Clinical Manifestations

When immune recovery causes unmasking of subclinical TB, the severity of clinical manifestations at a given anatomic site is likely to depend on the interrelationship between two main variables: the mycobacterial antigen load and the rate, intensity, and quality of immune recovery (Figure 3). In those in whom immune function remains very poor, TB is likely to remain subclinical despite an increasing mycobacterial load. Those patients with an intermediate rate of immune recovery would tend to develop clinical disease with a relatively "normal" presentation. In contrast, we propose that patients with dysregulated immune recovery are the ones most likely to develop unusual and severe manifestations that characterize IRD. This would be most pronounced in those with a high mycobacterial load (Figure 3). Within this model, therefore, only a proportion of patients in whom ART causes the unmasking of TB in the initial months of ART would fulfill existing case definitions for IRD. With a disease that is as heterogeneous in its clinical presentation as TB, it will clearly be difficult to develop a robust case definition for these cases.

CLINICAL AND PROGRAMMATIC IMPLICATIONS

The high early burden of TB during ART highlights the fundamental problem of how to efficiently screen for TB among patients preparing for ART. The less adequate the pre-ART screening processes, the greater the burden of TB is likely to be after starting treatment. Optimal screening strategies need to be defined; it is possible that all patients entering ART programs in resource-limited settings should ideally undergo culture-based screening for TB regardless of the presence or absence of symptoms. The major need to upgrade laboratory facilities and expand availability of culture-based diagnosis in resource-limited settings has been recognized and more sensitive tools for TB diagnosis at the point of care are desperately needed (30).

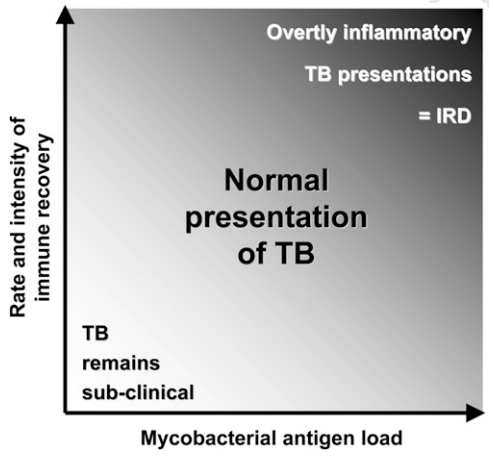

Figure 3. Progression of subclinical disease to symptomatic tuberculosis (TB) during early antiretroviral therapy (ART). This hypothetical conceptual diagram shows the interrelationship between *Mycobacterium tuberculosis* antigen load, rate, and intensity of immune recovery during early ART and the resulting clinical presentation of TB (*plot area*) in patients with subclinical TB at the time of ART initiation. Most cases of incident TB present with relatively "normal" clinical features, and TB immune reconstitution disease (TB IRD) forms only a subset of incident TB cases. TB IRD is most likely in patients with high antigen burden and dysregulated immune recovery, leading to development of exaggerated and overtly inflammatory manifestations of TB.

Although screening for TB among patients with advanced HIV-associated immunodeficiency is difficult, ART may paradoxically serve as a useful diagnostic tool, serving to unmask occult disease. Research is needed to determine the relative merits of delaying initiation of ART to permit full culture-based investigation for TB versus starting ART without delay if there is no initial evidence of TB. Because few of the cases of unmasking TB have increased clinical severity and yet delays in ART initiation may be associated with high mortality risk in this setting (51), we would not advocate a strategy of deferring ART while waiting for TB culture results in all patients.

Many in the field agree that management of those with moderate or severe TB-associated IRD should include corticosteroids (33–35). Pending the results of a randomized controlled trial in South Africa, however, the only evidence for this comes from case reports and expert opinion. In view of the seemingly normal clinical presentations of the large majority of cases of unmasking TB presenting in the initial months of ART, however, it seems likely that most patients who develop TB during early ART are unlikely to require adjunctive immunomodulatory therapy. On the basis of the same premise, there may be little merit in a strategy of using corticosteroids as prophylaxis against unmasking TB in high-TB-burden settings. Potential benefits, if any, may well be restricted to a small minority of patients and could be outweighed by adverse events (52).

Finally, it has been speculated that the unmasking of TB during the early months of ART may contribute to the overall burden of TB in resource-limited countries where ART is being rolled out (16). This is of course true and is an inevitable consequence of hundreds of thousands of lives being saved by expanding access to ART. However, the longer term burden of TB during ART (rather than that occurring in the initial months of treatment) is much more important with regard to TB control at the population level (9, 49, 53).

CONCLUSIONS AND SUGGESTED TERMINOLOGY

Using data and clinical experience from both low- and high-TB/HIV-burden countries, we propose that a spectrum of mechanisms underlies the development of TB during the first 3 months of ART. Much of this disease is likely to represent progression of subclinical TB that was present before ART initiation, either due to persisting immunodeficiency or due to unmasking during ART-induced immune recovery. The spectrum of effects of immune recovery on TB presentations in this period may include shortening of the time to development of symptoms (unmasking TB), increasing the rapidity of the initial onset of symptoms of TB, and increasing the intensity of clinical manifestations. These effects are likely to depend on the antigen load and the rate and intensity of immune recovery. Much remains to be learned from studies of the immunologic mechanisms underlying these phenomena.

Because it is presently not possible to clinically distinguish the underlying disease mechanisms, we suggest that cases of TB presenting during the initial months of immune recovery might collectively be referred to as "ART-associated TB." To refer to

the phenomenon whereby immune recovery triggers the presentation of TB, we suggest the term "unmasking" disease be used. Finally, in agreement with the existing literature, we suggest that only the subset of patients with exaggerated and overtly inflammatory manifestations of TB should be referred to as IRD. To enable case definitions for unmasking TB and TB IRD to be derived, further immunologic and clinical studies are needed to better characterize these phenomena.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Immune reconstitution inflammatory syndrome

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We read with interest Monika Müller and colleagues'[1] systematic review and meta-analysis of immune reconstitution inflammatory syndrome (IRIS) in the April, 2010, issue of *The Lancet Infectious Diseases*. Antiretroviral therapy (ART)-induced IRIS is a highly heterogeneous adverse effect arising in the initial months of treatment. The investigators should be congratulated for providing an important summary of IRIS frequency in patients with a range of HIV-associated diseases.

On assessing the data for the Review, some studies seem to be extreme outliers with unusually low frequencies of IRIS, which is striking. For example, the study by Wan Beom Park and colleagues[2] from South Korea shows a rate of only 2% for tuberculosis-associated IRIS. Similarly, our study[3] from Cape Town, South Africa, was reported as showing a rate of only 2% for cryptococcal-associated IRIS. Both of these results are well below the lower 95% credibility intervals of Müller and colleagues' meta-analysis. We are concerned that the data for ours and for Park and colleagues' study have been misinterpreted, and that two broad categories of IRIS, which should be considered as separate entities within such an analysis, have not been regarded.

Paradoxical IRIS is used to describe IRIS among patients who are already receiving treatment for an opportunistic disease, and in whom immune recovery after subsequent initiation of ART triggers the clinical deterioration of that disease during the initial months of treatment. In contrast, unmasking IRIS is used to denote the clinical event in which opportunistic disease, which was not present at the time of ART initiation, becomes clinically manifest because of ART-induced immune recovery. Although risk of paradoxical IRIS is strongly related to the timing of ART during treatment for the opportunistic disease,[4] the frequency of unmasking IRIS will vary with the prevalence of the opportunistic disease in any given setting, and with the efficiency of screening before ART.[5], [6] Tuberculosis and cryptococcal disease can both be associated with either form of IRIS. Because the denominators that are used to calculate the frequencies of these two forms of IRIS are mutually exclusive, the calculation of pooled summary estimates from data that include both forms of IRIS makes little sense. We suspect that this explains why the results from Park and colleagues, and from our study, seem to be outliers in Müller and colleagues' analysis, and we are concerned that this irregularity might have particularly undermined the summary estimate of the meta-analysis for the frequency of cryptococcal IRIS.

In their meta-analysis of cryptococcal IRIS, Müller and colleagues sourced data from six studies, five of which relate exclusively to the risk of paradoxical IRIS in patients with known cryptococcal disease before starting ART. In these five studies, 67 cases of IRIS were reported among 245 patients with a crude frequency of 27·3%. However, data were also included from our cohort in Cape Town, South Africa.[3] In this study we

described six cases of paradoxical-cryptococcal IRIS among 18 patients with a pre-existing diagnosis of cryptococcal meningitis, giving a frequency of 33% (95% CI 16–56). This proportion is entirely consistent with the other five studies, and including these cases in this form would have been entirely appropriate. However, we also reported three cases of unmasking-cryptococcal IRIS among the remaining cohort of 416 patients who did not have pre-existing cryptococcal disease. Unfortunately, Müller and colleagues have not distinguished between paradoxical and unmasking forms of disease, and simply reported an overall rate of 2% for cryptococcal IRIS in the total cohort of 434 patients. The study therefore seems to be an extreme outlier within the data of the meta-analysis. Both the numerator and denominator, which we used to calculate the event frequency are entirely different to those obtained from the remaining five studies and, therefore, the meta-analysis summary estimate from the total of six studies is unfortunately flawed. The effect of this flaw will have been to underestimate the true frequency of paradoxical-cryptococcal IRIS.

A further consequence of the failure to distinguish between paradoxical and unmasking forms of IRIS is that the timing of ART in patients with tuberculosis or cryptococcal meningitis has not been considered. This factor is a crucial determinant of the risk of paradoxical IRIS[4] and might partly explain the considerable heterogeneity in rates between studies. Clinicians can control this key variable, and thus it is the subject of several ongoing randomised trials. Notwithstanding these limitations, our own experience in this specialty is consistent with the important findings by Müller and colleagues that cryptococcal IRIS is the form of IRIS most strongly associated with mortality.[7], [8]

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Impact of HIV Infection on the Epidemiology of Tuberculosis in a Peri-Urban Community in South Africa: The Need for Age-Specific Interventions

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(See the editorial commentary by Whalen on pages 1048–50)

Background. In August 2005, the World Health Organization declared the tuberculosis (TB) epidemic in Africa to be a regional emergency. Current TB-control measures are failing, largely as a result of the human immunodeficiency virus (HIV) epidemic. Evaluation of additional control interventions requires detailed understanding of the epidemiological relationship between these diseases at the community level.

Methods. We examined age- and sex-specific trends in TB notifications and their association with the prevalence of HIV infection in a peri-urban township in South Africa during 1996–2004. Denominators for TB notifications were derived from population census data. The local TB-control program used the World Health Organization directly observed treatment, short-course (DOTS) strategy.

Results. TB notification rates increased 2.5-fold during the period, reaching a rate of 1468 cases per 100,000 persons in 2004 ($P = .007$, by test for trend); the estimated population prevalence of HIV infection increased from 6% to 22% during the same period. After stabilization of prevalence of HIV infection, the TB notification rate continued to increase steeply, indicating ongoing amplification of the TB epidemic. In 2004, at least 50% of children aged 0–9 years who developed TB were HIV infected. Annual TB notification rates among adolescents increased from 0 cases in 1996–1997 to 436 cases per 100,000 persons in 2003–2004, and these increases were predominantly among female. However, 20–39-year-old persons were affected most, with TB notification rates increasing from 706 to 2600 cases per 100,000 persons among subjects in their 30s. In contrast, TB rates among persons aged >50 years did not change.

Conclusions. HIV infection is driving the TB epidemic in this population, and use of the DOTS strategy alone is insufficient. TB notifications have reached unprecedented levels, and additional targeted, age-specific interventions for control of TB and HIV infection in such populations are needed.

Annual tuberculosis (TB) incidence rates have increased by 2- to 3-fold in many countries in sub-Saharan Africa since 1990 [1]. The African continent, which contains just 11% of world's population, now accounts for 27% of the global burden of TB and 30% of TB-related deaths; an estimated 2.4 million new TB cases and 540,000 TB-related deaths occur in Africa annually [1]. The World Health Organization (WHO) TB-control strategy, which is based on the directly observed treatment, short course (DOTS) strategy, has failed to contain the African TB epidemic, primarily because of the effects of the HIV epidemic in the region. In August 2005, the WHO Committee for Africa declared the TB epidemic to be an African regional emergency [2]. Rapid escalation of the disease rate in the region is undermining progress towards the Millennium Development Goals for TB control and was deemed as mandating "urgent and extraordinary actions" [2].

An interaction between the HIV and TB epidemics has been well established by demonstration of increased TB case load in populations affected by HIV infection; an increased prevalence of HIV infection among persons with TB, compared with the general population;

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and progressively increasing risk of TB with advancing immunodeficiency [3–5]. The impact of HIV infection is accentuated in populations with preexisting high TB case rates [5]. Moreover, the impact on TB notification rates may increase during the course of a rapidly maturing HIV epidemic as the proportion of patients with advanced immunodeficiency increases [3]. Thus, the relationship between HIV and TB epidemics in a community is likely to evolve over time.

Existing data on the population-level interaction between the TB and HIV epidemics in sub-Saharan Africa are derived largely from national or regional data [5]. Such data are often incomplete and do not define the relationship within individual communities bearing the brunt of disease. Insights into the dual epidemic have also been derived from studies of rural districts or unusual populations, such as gold miners [6–10]. However, neither population is likely to reflect the situation in urban and peri-urban communities.

We investigated epidemiological changes in TB notifications and the prevalence of HIV infection during 1996–2004 in a well-defined, peri-urban community in the Western Cape, South Africa. The study objective was to document the epidemiological association of these 2 epidemics in a community during a period in which the prevalence of HIV infection rapidly increased. Such information is needed to provide a scientific basis for any modification of present TB-control strategies.

METHODS

Study population. We studied a peri-urban township near Cape Town, South Africa, which was established in 1992 and has grown to its current population size of ~13,000 people. The township is home to an almost exclusively African population, and the majority of persons have low socioeconomic status. Unemployment rates exceed 50%, and housing predominately consists of closely aggregated, informal structures with high levels of overcrowding. The township is geographically clearly demarcated and constitutes a well-defined population for research studies and community health interventions. This research was approved by the Research Ethics Committee of the University of Cape Town.

Health services. The study community is served by a single government primary health care clinic with a dedicated TB service. All patients with TB in the community are treated at this facility. The program adhered to the South African National TB Control Programme guidelines throughout the study period and included the WHO DOTS strategy. DOTS coverage in this community was complete, and cure rates for smear-positive disease exceeded 80% in the district [11]. All sputum testing was performed at the National Health Laboratory Services facilities in Cape Town.

Case definitions. Pulmonary sputum-positive TB was diagnosed on the basis at least 1 positive sputum culture of *Mycobacterium tuberculosis* or 2 sputum smears containing acid-fast bacilli in the context of a compatible clinical illness. Pulmonary sputum-negative TB was diagnosed on the basis of negative smears and cultures for *M. tuberculosis* in the context of clinically and radiologically compatible illness of at least 3 weeks' duration that did not respond to administration of simple antibiotic treatment but that responded to subsequent antituberculosis treatment. Diagnosis of extrapulmonary TB was based on a combination of clinical, radiological, and histopathological findings, as well as a response to antituberculosis treatment.

Data sources. Numbers of TB notifications, demographic characteristics, history of previous TB, sputum microbiologic test findings, and TB classification data were obtained from the community TB clinic register. Demographic data for the community were derived from the 1996 South African national census and from a household census performed in 2004 as part of ongoing health research. Prenatal HIV prevalence data were collected within the local Prevention of Mother to Child Transmission program. The prevalence of HIV infection among patients who had TB diagnosed was obtained from the results of voluntary counseling and testing routinely performed at the TB clinic in 2004 and from re-treatment cases tested in 2003–2004.

Estimates of the prevalence of HIV infection. Age-specific estimates of the prevalence of HIV infection were made using the Actuarial Society of South Africa 2002 demographic model for the African population. This model estimates the prevalence of HIV infection in South Africa using prenatal surveys and demographic data. The assumptions and results of the model have been described elsewhere [12, 13], and this model was validated for use in this community using a cross-sectional survey of 6% of the population in 2005.

Data analysis. Denominators for rate calculations were based on extrapolations of 1996 and 2004 population census data, assuming a linear trend in age- and sex-specific distributions over the study period. TB notification rates, TB re-treatment rates, and age- and sex-specific rates of TB were calculated using the data from the TB register and population census data. Ninety-five percent CIs for rates are based on Poisson-distributed standard errors. Trend analyses were conducted using Cuzick's nonparametric test for trend [14]. Changes over time in the median age of subjects with TB diagnoses were compared using the Wilcoxon rank sum test. All tests were 2-sided at $\alpha = 0.05$.

RESULTS

TB notification rates and prevalence of HIV infection. During 1996–2004, a total of 968 cases of TB were diagnosed. The annual TB notification rate increased 2.5-fold over the

study period, reaching 1468 cases per 100,000 persons in 2004 (table 1). Rates of TB re-treatment also increased to >20% of notifications in 2003–2004.

The estimated prevalence of HIV infection in the community increased from 6% to 22% (table 1). In 2004, among persons aged >15 years, 98 (59%) of 166 persons who provided TB notifications were HIV seropositive, whereas 30% were HIV seronegative, and 11% had not been tested. On the basis of these data, the TB notification rate among HIV-infected individuals was calculated to be 4381 cases per 100,000 persons (95% CI, 3570–5313 cases per 100,000 persons), and the rate among HIV-uninfected individuals was 656 cases per 100,000 persons (95% CI, 486–866 cases per 100,000 persons). Even if persons who were not tested in 2004 were assumed to be HIV seronegative, a statistically significant increase in TB notification rates among HIV-uninfected individuals in this community could not be demonstrated (data not shown). Among patients who underwent re-treatment, cure of the previous episode of TB was microbiologically confirmed among 32 patients in 2003–2004. Of these 31 tested patients, 27 (87%) were found to be HIV seropositive.

Spectrum of TB. Of the 968 TB cases diagnosed, 450 (46.5%) were pulmonary sputum positive, 247 (25.5%) were pulmonary sputum negative, 140 (14.5%) involved extrapulmonary disease (predominantly lymph node), and 108 (11.2%) were primary disease among children. Sputum test results were missing for 23 cases of pulmonary disease, representing 2.3% of all TB notifications.

The spectrum of TB diagnoses changed over the course of the study period (figure 1). The notification rate of sputum-positive TB cases increased 1.9-fold, from 326 cases per 100,000 persons (95% CI, 181–471 cases per 100,000 persons) in 1996 to 617 cases per 100,000 persons (95% CI, 481–752 cases per 100,000 persons) in 2004 ($P < .01$, by test for trend). However, increases in the rates of extrapulmonary and sputum-negative pulmonary disease were proportionately greater, increasing 4.3-fold from 167 cases per 100,000 persons (95% CI, 63.8–271.0 cases per 100,000 persons) in 1996 to 719 cases per 100,000 persons (95% CI, 572–865 cases per 100,000 persons) in 2004 ($P < .01$, by test for trend).

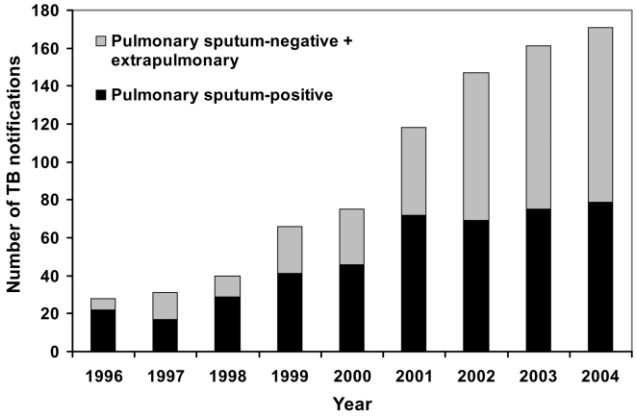


Figure 1. Annual numbers of tuberculosis (TB) notifications, stratified by site of disease and sputum microbiologic test findings. Children with diagnoses of primary TB were excluded.

TB among adults. Predominantly young people lived in this community, with the modal age lying in the 20–29-year-old age range; 88% of the population were <39 years of age. Comparison of census data from 1996 with data obtained in 2004 showed that the age-sex composition of this population had not changed significantly. Subsequent analysis of the age-specific distribution of TB notifications during 1996–2004 revealed a marked increase in the number of notifications among 20–39-year-old persons (figure 2*A*). This pattern was similar to increases in the estimated age-specific prevalence of HIV infection in this population during the same period (figure 2*B*). Thus, increases in the prevalence of HIV infection and the

Table 1. Tuberculosis (TB) notification rates and the prevalence of HIV infection in a peri-urban community in the Western Cape, South Africa, 1996–2004.

Year	No. of TB notifications	Population size	TB notification rate, cases/100,000 persons[a]	TB re-treatment rate, %[b]	Estimated prevalence of HIV infection, %
1996	32	5518	580	3	6.3
1997	42	6429	653	21	8.9
1998	67	7339	913	7	11.6
1999	74	8250	897	20	14.2
2000	90	9161	982	17	16.5
2001	142	10,071	1410	15	18.4
2002	150	10,982	1366	18	19.9
2003	175	11,892	1472	22	21.1
2004	188	12,803	1468	24	21.9

[a] $P = .007$, by test for trend.
[b] $P = .073$, by test for trend.

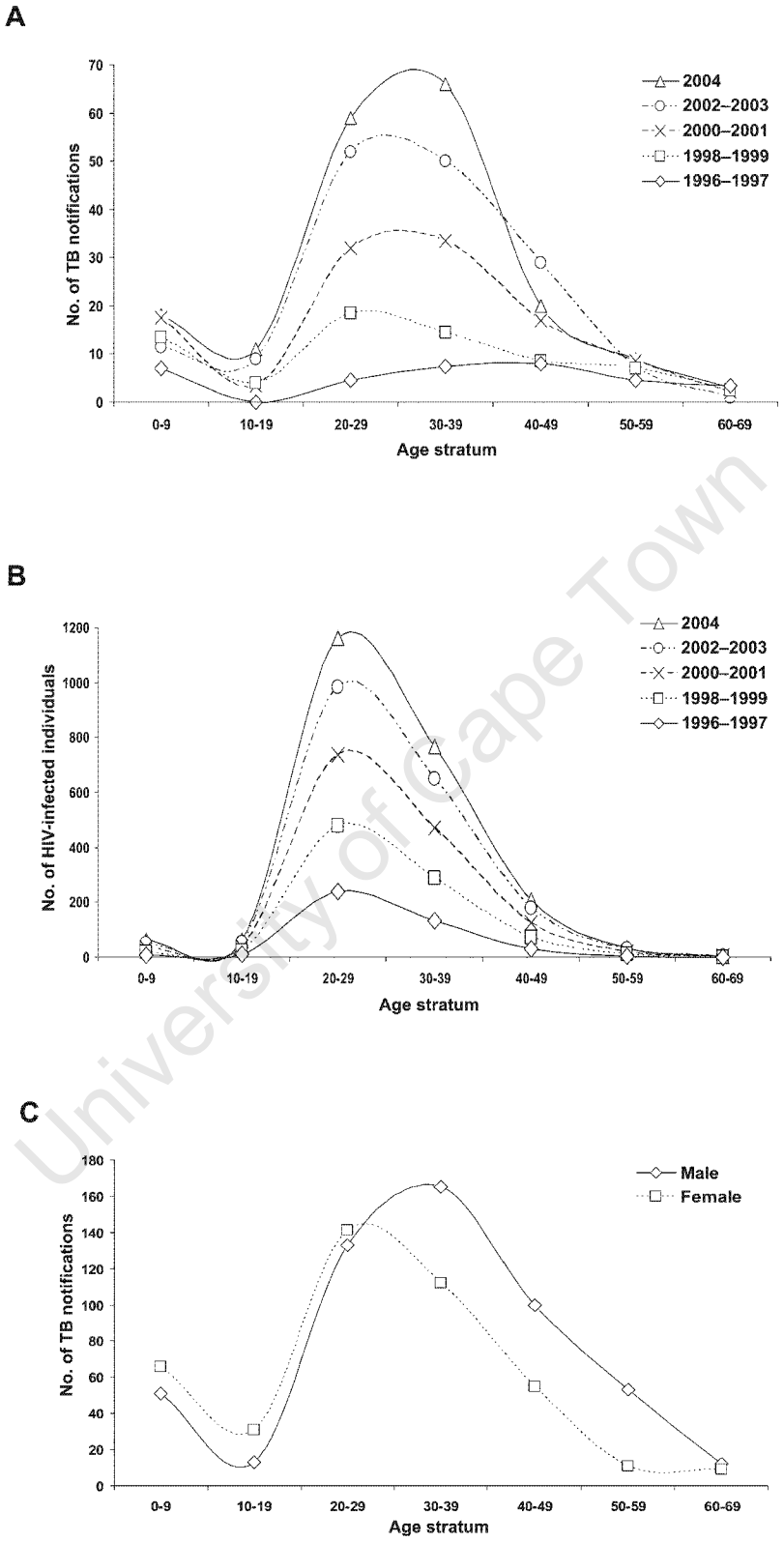


Figure 2. *A,* Changes in the number of tuberculosis (TB) notifications, stratified by age, 1996–2004. *B,* Estimated numbers of HIV-infected individuals, stratified by age, 1996–2004. *C,* Total number of TB notifications, stratified by age and sex, 1996–2004. Changes over time are predominantly seen in the 20–39-year-old age group. This shows that TB affects more men than women, with the notable exception of the adolescent age group.

escalating burden of TB were both concentrated among young adults.

During 1996–2004, the median age of patients with TB (excluding those aged <10 years) decreased from 43 years to 32 years ($P < .001$). Additional comparison of age- and sex-specific notifications showed that the modal age group for patients with TB was 20–29 years for women and 30–39 years for men (figure 2*C*).

Although the distribution of age-specific TB notification rates observed during 1996–1997 conformed to that observed historically in this region [15], the distribution changed substantially over the study period. The highest TB notification rates in 2003–2004 occurred among adults aged <50 years (figure 3), and the greatest increase in notification rates was among persons aged 30–39 years, reaching 2600 cases per 100,000 persons in 2004. However, no significant increase in rate occurred among those aged ⩾50 years.

TB among adolescents and children. TB was notified for 44 adolescents aged 10–19 years (31 of whom were female, and 13 of whom were male). Although no cases occurred in 1996–1997, the TB notification rate among adolescents steadily increased from 1998 onwards, particularly among female subjects (figure 2*A* and 2*C*), to reach an average annual rate of 436 cases per 100,000 persons in 2003–2004 (figure 3). Of 11 TB cases notified in 2004, 5 involved persons aged <16 years who had not undergone HIV testing. Among the remaining 6 adolescents tested, 3 were HIV seropositive.

The increase in TB notification rates among children aged 0–9 years was not statistically significant. However, 18 cases were diagnosed in 2004, and of 13 children tested, 9 (50% of total cases) were found to be HIV infected.

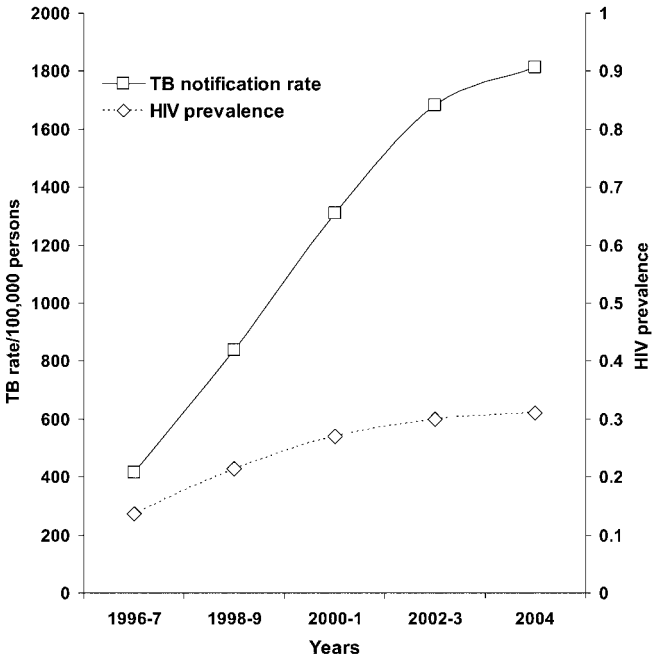
Temporal association between HIV and TB epidemics. We next analyzed the rates of these 2 diseases among persons aged 20–39 years in whom both epidemics were concentrated (figure 4). Major increases in the prevalence of HIV infection occurred before 2000, and yet a high rate of increase in the TB notification rate continued thereafter. With data from 1996–1997 used as baseline values, we calculated the increases in the TB notification rate per unit increase in the prevalence of HIV infection. For each 1.0% increase in the prevalence of HIV infection, TB notification rates increased by 54.7 cases per 100,000 persons in 1998–1999 and by 80.6 cases per 100,000 persons in 2004. Thus, increases in the prevalence of HIV infection were associated with ongoing amplification of the TB epidemic several years later.

DISCUSSION

To our knowledge, this is the first community-based study from sub-Saharan Africa to characterize in detail the epidemiological associations over time between rapidly evolving epidemics of TB and HIV infection. The 2.5-fold increase in overall TB notification rates culminated in annual rates exceeding 1400 cases per 100,000 persons, ~9-fold higher than the TB notification rate for sub-Saharan Africa and 280-fold greater than that for the United States [1]. A striking temporal association between the evolving age-specific burdens of TB and HIV infection was demonstrated. Despite the existence of a well-run TB-control program, the DOTS strategy alone is failing to control TB in this population, and additional measures are urgently needed. These data provide an epidemiological basis with which to target interventions.

2003–2004	731	436	1369	2599	2824	1856
1996–1997	582	0	245	706	1405	1708

Figure 3. Age-specific tuberculosis (TB) notification rates (95% CI) in 1996–1997, compared with rates in 2003–2004. Rates significantly increased among 10–49-year-old persons, with the greatest rate increase among those aged 30–39 years.



Increase in TB notification rate (per 100,000 persons) per 1.0% rise in HIV prevalence	-	54.7	67.3	77.8	80.6

Figure 4. Changes in age-specific tuberculosis (TB) notification rates and the prevalence of HIV infection among persons aged 20–39 years, 1996–2004. With data from 1996–1997 used as baseline values, increases in the TB notification rate per unit increase in the prevalence of HIV infection are tabulated below the graph.

The strength of this study lies in fact that this clearly defined community was well characterized by total-population census surveys at the beginning and the end of the study period. This permitted calculation of disease rates, taking into account the changing demographic profile of the community. Moreover, the community is served by a single health center, where all patients with TB in this community are treated. Thus, data completeness is likely to be high. Trends in TB incidence in this community before the HIV epidemic are not known, and we cannot exclude other factors in addition to HIV infection that may have contributed to the increasing burden of TB. However, TB notification rates in the Western Cape were relatively stable in the 1980s, before the advent of the HIV epidemic in South Africa. Whether these data can be generalized is unknown, and the findings may apply only to similar communities with a high burden of TB and HIV infection.

Annual TB notification rates exceeding 1400 cases per 100,000 persons are almost unparalleled in the era of modern antituberculosis treatment, except for rates reported among the Inuit population of Northern Canada in the early 1960s [16]. Three key factors probably underlie the explosive TB epidemic in this and similar communities: the high pre-existing TB notification rate, the rapid increase in the prevalence of HIV infection, and the population composition, in which 20–40-year-old persons who bear the brunt of these 2 diseases are disproportionately represented. The strongest factor pointing towards HIV infection as the principal cause underlying the increasing burden of TB was the temporal association between the evolving age-specific burdens of the 2 epidemics (figure 2*A* and 2*B*). Other factors corroborating this association were the disproportionate increases in the rates of extrapulmonary and pulmonary sputum-negative TB and the high prevalence of HIV infection among patients with TB.

The epidemiology of TB in this community has changed profoundly over a short period of time. Historically, TB notification rates in this community have been highest among persons >60 years of age [15]. However, in 2003–2004, the rates were highest among persons aged 30–49 years (figure 3), indicating a major shift in the burden of disease to individuals in the economically productive age groups who also have the most dependents. A particularly concerning finding was the burden of TB among adolescents, especially girls, with TB rates among 10–19-year-old adolescents reaching 436 cases per 100,000 persons in 2004. We have since documented that the

prevalence of HIV infection among adolescents aged 10–19 years in this community was 7% among male subjects and 11% among female subjects in 2004 (unpublished data), indicating that increases in the burden of TB in this age group were very likely to be, at least in part, attributable to HIV infection.

The risk of TB increases with advancing immunodeficiency [3, 4], so as the HIV epidemic in a community matures, the burden of HIV-associated TB may be expected to increase, even after the prevalence of HIV infection has stabilized. This is likely to explain why the major increases in the prevalence of HIV infection in this community occurred before 2000, yet TB notification rates continued to increase steeply thereafter (figure 4). These data suggest that ongoing amplification of the TB epidemic continues for several years as a result of earlier increases in the prevalence of HIV infection.

Although increases in the number of notifications were more pronounced for less infectious types of TB (i.e., pulmonary sputum-negative and extrapulmonary TB), the number of notifications of infectious, sputum-positive disease also increased substantially (figure 1). The secondary impact of HIV-associated burden of sputum-positive TB in the general community remains incompletely defined [7, 9, 17], but we were unable to demonstrate a statistically significant increase in rates among HIV-uninfected individuals. Moreover, TB notification rates among persons aged ⩾50 years, among whom the prevalence of HIV infection is low, did not increase (figure 3). HIV-associated sputum-positive disease clearly does not cause a proportionate increase in transmission within the general community. This is almost certainly related to the fact that HIV-associated TB becomes symptomatic more quickly and is diagnosed earlier than disease in HIV-uninfected individuals [6], diminishing the period for potential transmission.

Although the DOTS strategy is central to the WHO policy for TB control, the explosive increase in TB notifications in this community and others like it show that the DOTS strategy alone is not sufficient, and additional measures are required. We suggest that these strategies may be targeted in various ways on an age-specific basis among children, adolescents, those aged 20–49 years, and those aged ⩾50 years. A substantial proportion of children who developed TB in 2004 were HIV infected. Thus, prevention of mother-to-child transmission services need to be strengthened, and voluntary counseling and testing for HIV in prenatal clinics and access to antiretroviral treatment must be provided for HIV-infected pregnant women. Secondly, administration of isoniazid prophylaxis to household contacts of adults with TB needs to be more rigorously implemented.

The escalating burden of TB and HIV infection among adolescents, among whom TB was previously rare, is of great concern. Schools in this and similar communities across Africa are extremely crowded and may provide a key site of TB transmission. Moreover, in South Africa, the prevalence of HIV infection is high among teachers [18], who may, therefore, represent an important source of TB transmission. Therefore, we suggest that TB and HIV diagnostic and prevention services for adolescents and teachers should be introduced into schools.

Annual TB notification rates among persons aged ⩾50 years did not increase but remained ~1800 cases per 100,000 persons (figure 3). The major burden of disease in this age group was among men who were unlikely to be HIV infected (figure 2*B* and 2*C*). Because HIV-uninfected individuals with TB are more infectious [19], this age group may represent a key source of TB transmission and may be targeted by intensified case-finding.

Most of the increasing burden of TB occurred among 20–49-year-old persons. TB and HIV interventions need to be rigorously implemented in health care facilities, and provision of such services in workplaces may also facilitate access for this age group. In this community, HIV infection is frequently diagnosed once individuals have presented with TB; strategies to diagnose HIV infection before the development of TB are urgently needed. This mandates intensification of voluntary counseling and testing in prenatal, sexual health, and family-planning clinics and whenever individuals interface with the health care system.

In addition to the DOTS strategy, antiretroviral therapy may play an important adjunctive role in addressing this TB epidemic. Antiretroviral therapy reduces TB risk by 70%–90% in treated cohorts over 1–2 years of follow-up [4, 20]. Voluntary counseling and testing among patients with TB on an opt-out basis may be accompanied by use of antiretroviral therapy among all persons who are found to be HIV seropositive. The majority of such patients here and elsewhere fall within the eligibility criteria for antiretroviral therapy [21, 22]. This intervention may reduce both the rate of new cases of TB, as well as the high TB recurrence rate. However, there is concern that, although antiretroviral therapy is very effective in reducing an individual's risk of developing TB per unit time, the lifetime risk of disease may not decrease [20, 23]. Thus, the overall burden of TB in the community may not substantially diminish, despite good antiretroviral coverage. Other adjunctive interventions that may also be important include use of primary and secondary isoniazid prophylaxis [24, 25].

In conclusion, we have carefully documented the differential impact of HIV infection on TB notifications in age- and sex-specific groups in this community. These data show that additional interventions may need to be targeted in an age-specific manner to address this regional emergency.

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Undiagnosed Tuberculosis in a Community with High HIV Prevalence

Implications for Tuberculosis Control

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Background: **Although failure of tuberculosis (TB) control in sub-Saharan Africa is attributed to the HIV epidemic, it is unclear why the directly observed therapy short-course (DOTS) strategy is insufficient in this setting. We conducted a cross-sectional survey of pulmonary TB (PTB) and HIV infection in a community of 13,000 with high HIV prevalence and high TB notification rate and a well-functioning DOTS TB control program.**

Methods: **Active case finding for PTB was performed in 762 adults using sputum microscopy and *Mycobacterium tuberculosis* culture, testing for HIV, and a symptom and risk factor questionnaire. Survey findings were correlated with notification data extracted from the TB treatment register.**

Results: **Of those surveyed, 174 (23%) tested HIV positive, 11 (7 HIV positive) were receiving TB therapy, 6 (5 HIV positive) had previously undiagnosed smear-positive PTB, and 6 (4 HIV positive) had smear-negative/culture-positive PTB. Symptoms were not a useful screen for PTB. Among HIV-positive and -negative individuals, prevalence of notified smear-positive PTB was 1,563/100,000 and 352/100,000, undiagnosed smear-positive PTB prevalence was 2,837/100,000 and 175/100,000, and case-finding proportions were 37 and 67%, respectively. Estimated duration of infectiousness was similar for HIV-positive and HIV-negative individuals. However, 87% of total person-years of undiagnosed smear-positive TB in the community were among HIV-infected individuals.**

Conclusions: **PTB was identified in 9% of HIV-infected individuals, with 5% being previously undiagnosed. Lack of symptoms suggestive of PTB may contribute to low case-finding rates. DOTS strategy based on passive case finding should be supplemented by active case finding targeting HIV-infected individuals.**

Keywords: African community; case finding; HIV infection; incidence and prevalence; pulmonary tuberculosis

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The complex interaction between the dual HIV and TB epidemics at a community level is underreported.

What This Study Adds to the Field

Pulmonary tuberculosis is common in HIV-infected individuals. Specific active case finding strategies need to be targeted at HIV-infected individuals.

Present tuberculosis (TB) control strategies based on directly observed therapy short-course (DOTS) strategy is insufficient to contain TB in sub-Saharan Africa, largely because of the parallel epidemic of HIV in the region (1). An estimated 2.4 million new TB cases and 540,000 TB-related deaths now occur in sub-Saharan Africa annually. Consequently, in August 2005, the World Health Organization declared the epidemic in Africa to be a regional emergency (2).

The DOTS strategy has been shown to be effective in many settings, particularly where levels of HIV are relatively low (3–5). However, the reasons for increasing TB rates where HIV is prevalent have not been clearly defined. Failure of TB control may in part be due to poor implementation of the DOTS strategy because of lack of infrastructure or adequate resources (6). However, TB control may be less effective in communities with high HIV prevalence because of alterations in patterns of transmission or disease presentation. By examining where DOTS has been less successful (7), we may understand the reasons for the breakdown in TB control efforts and suggest modifications and improvement in current approaches and design new control strategies.

The interactions between HIV and TB are complex; however, understanding the interplay between these two epidemics is particularly important in countries of southern Africa, where HIV has fueled an unprecedented increase in TB notification rates (8). Because infectivity decreases rapidly after initiation of anti-TB therapy (9), TB control is based on rapid identification and treatment of infectious cases, thereby reducing the mean duration of infectiousness and the resultant number of secondary infections (10). However, HIV may affect TB case finding due to atypical presentations of TB (11–16) and the presence of

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coinfections, which may mimic the symptoms of TB. HIV-mediated immune deficiency also increases risk for development of disease (17), and the vulnerable population increases during the course of a maturing HIV epidemic as the proportion of those with advanced immune deficiency increases (18, 19).

We have previously reported an increasing TB notification rate in a community with high HIV and TB prevalence despite implementation of a DOTS TB control program with good outcome measures (20). In this population of approximately 13,000 individuals, data collected over 10 yr have shown a 2.5-fold increase in TB notifications, to unprecedented levels exceeding 1,400 of 100,000, coincident with HIV prevalence in the community increasing from 6 to 22% (20). In the present study, we conducted a cross-sectional, population-based survey of both HIV and TB prevalence to estimate the burden of undiagnosed TB among HIV-infected people in this community. To avoid selection bias, all individuals in the study sample were screened for TB without any preselection on the basis of symptoms. In conjunction with notification data, these data allow the estimation of case-finding rates under the local DOTS program and the population burden of undiagnosed TB disease. Some of the results of this study have been previously reported as an abstract (21).

METHODS

Study Community

The study community comprised 13,000 black African individuals living mainly in shacks in a high-density residential area sited within well-demarcated boundaries, where unemployment exceeds 50% (20). The estimated mid-2005 adult population (> 14 yr) was derived from a household survey performed in December 2004, adjusted for population growth. There is a single public-sector primary care clinic, which incorporates voluntary HIV counseling and testing, and a TB clinic for the diagnosis and management of TB in accordance with the South African national TB control program protocols.

TB and HIV Prevalence Survey

Between February and June 2005, a total of 1,150 adults included in the 2004 census were selected using simple random sampling. This sample size was chosen to calculate an anticipated prevalence of TB of 2.5%, with precision of 1.0%, and with corrections for anticipated nonresponse (22). An initial home visit confirmed 971 of the selected adults were living permanently on the identified plots, and these individuals were invited to participate in the study. Of these, 762 adults (78%) consented to participate in the study, 61 were unable to be contacted after five home visits, and 148 declined to participate.

Participants were requested to bring an early-morning sputum sample to the study site. At the study visit, a second saline nebulized sputum sample was collected and an oral transudate was taken for anonymous linked HIV testing; HIV test results were available for 758 individuals (two patients declined testing and two specimens were unsuitable for analysis). Voluntary counseling, testing, and disclosure were offered to all participants wishing to know their HIV status. HIV counseling, care, treatment, and prevention services are run from the primary health care clinic on site. A structured questionnaire investigating participant demographic characteristics, TB symptoms (cough, loss of appetite, weight loss, and night sweats), risk factors for TB (including housing, alcohol, smoking, recreational drug use, incarceration, previous contact with health services, time away from community, and employment history), and risk factors for HIV infection (including number of sexual partners and condom use) was completed. Questionnaires were administered in participants' home language (predominantly isiXhosa) by trained interviewers who used standardized isiXhosa phrasing for all questions. All interviews took place in a private room within the clinic. Draft questionnaires, including translated versions, were piloted before the study began to ensure appropriate translation and participant comprehension. All participants gave written, informed consent. The study received approval from the Research Ethics Committee of the University of Cape Town.

Case Definitions for TB Disease

TB definitions used for notification data were as defined by the South African TB Control Program (23). Definitive prevalent smear-positive TB was defined as a positive sputum smear confirmed with a second positive smear or culture of *Mycobacterium tuberculosis*; and smear-negative pulmonary TB (PTB) was defined by two positive cultures of *M. tuberculosis* with confirmed identical spoligotype patterns.

TB Incidence and Treatment Outcomes

TB is a notifiable condition in South Africa, and each TB clinic is required to maintain and report TB statistics, including patient demographics, TB diagnostic criteria, treatment regimen prescribed, results of laboratory monitoring, voluntary HIV counseling and testing results, and treatment completion and interruptions.

The adult TB case notification rate was determined as the number of cases notified and initiating TB therapy during the 2005 calendar year. Program performance criteria were extracted from the TB register for all patients commencing therapy from 2002 to 2004.

Laboratory Procedures

Sputum smears were examined for acid-fast bacilli (AFB) using the auramine O fluorescent stain. Mycobacterial growth indicator tubes (Becton-Dickinson, Sparks, MD) were inoculated according to the manufacturer's instructions and incubated for 8 wk before being recorded as negative. Positive cultures were screened for the presence of AFB by Ziehl-Neelsen staining, and identified as *M. tuberculosis* complex by inhibition of growth on p-nitrobenzoic acid. A polymerase chain reaction assay specific for *M. tuberculosis* (24) was performed on those cultures that had AFB visible on microscopy, but were contaminated and could thus not be identified using the p-nitrobenzoic acid test. DNA isolated from all *M. tuberculosis* cultures underwent spoligotyping.

An oral mucosal transudate for HIV testing was collected using the OraSure (OraSure Technologies, Bethlehem, PA) oral fluid collection device. The Vironostika Uni-Form II (bioMérieux, Marcy-l'Etoile, France) HIV-1 and HIV-2 plus O ELISA test was used to test for HIV-1 and HIV-2 antibodies.

Statistical Methods

Data were analyzed using Stata version 9.0 (StataCorp, College Station, TX). Notification rates are based on the mid-2005 population estimates. Median time receiving treatment in the TB program was calculated from date of commencing treatment to date of completion, death, and loss to follow-up, or transfer out of the program. Kaplan-Meier mortality proportions at 6 mo were estimated from the start of treatment to death, treatment completion, loss to follow-up, or transfer. Because the notification data come from routine service delivery where HIV testing is optional, test results were available for only 81% of notified PTB cases; for subsequent calculations, we made a conservative assumption that the prevalence of HIV in notified PTB cases of unknown HIV status was similar to the observed prevalence in notified cases with HIV data available. The mean time that PTB cases spent in the community before diagnosis and treatment (for both smear-positive disease and all PTB) was estimated as the observed PTB prevalence divided by the incidence of TB treatment (notification data). The time before treatment was corrected for the excess mortality associated with HIV infection in individuals with TB (as observed in the community DOTS program between 2002 and 2004). In turn, the total person-time (i.e., the time that different types of PTB cases spent in the community before diagnosis) is estimated as the product of the number of affected individuals in the community and the mean time before diagnosis. Case-finding proportions were calculated as the quotient of the prevalence of treated PTB divided by the sum of the prevalence of treated and the prevalence of untreated PTB. Exact 95% confidence intervals (CI) are based on the hypergeometric distribution (25). We evaluated risk factors for TB using Fisher's exact tests, and Mann-Whitney U tests were used to compare proportions and medians, respectively; all statistical tests were two-sided at $\alpha = 0.05$.

TABLE 1. 2005 ADULT PULMONARY TUBERCULOSIS TREATMENT NOTIFICATION RATES PER 100,000 PER ANNUM, CALCULATED FOR TOTAL ADULT POPULATION (n = 10,408) AND HIV-POSITIVE (n = 2,432) AND HIV-NEGATIVE (n = 7,976) POPULATIONS

	Adult Population (*95% CI*)	HIV-positive Adults (*95% CI*)	HIV-negative Adults (*95% CI*)
Pulmonary tuberculosis case notifications	1,931 (1,676–2,214)	5,140 (4,296–6,093)	953 (751–1,191)
Sputum smear–positive tuberculosis	1,307 (1,097–1,544)	3,248 (2,580–4,032)	715 (542–925)
Sputum smear–negative tuberculosis	625 (482–795)	1,891 (1,388–2,515)	238 (143–372)

Definition of abbreviation: CI = confidence interval.

HIV test results were available for only 81% of notified PTB cases; a conservative assumption has been made that the prevalence of HIV in notified PTB cases of unknown HIV status is similar to the observed prevalence in notified cases with HIV data available.

RESULTS

TB Notifications 2005

The mid-2005 adult population was projected to be 10,408 with an HIV seroprevalence of 23% using existing data (20). Thus, approximately 8,000 HIV-negative and 2,400 HIV-infected individuals constituted the study population. A total of 259 TB cases were notified in adults over the age of 14 yr and 201 (78%) of these were categorized as PTB. The direct sputum smear–positive and smear–negative adult PTB treatment notifications, stratified by HIV status, are shown in Table 1. HIV testing was performed in 81% of notified TB cases, with 66% testing positive. The HIV-related odds ratio (OR) for smear-positive TB was 4.7 (95% CI, 3.3–6.7) and for smear-negative TB was 8.1 (95% CI, 4.7–14.7).

In the 2002–2004 performance criteria for the TB control program among HIV-negative and HIV-positive individuals, treatment completion was obtained in 84 and 73%, mortality in 3 and 13%, transfer out in 9 and 12%, and treatment interruption occurred in 4 and 1%, respectively. The median length of TB treatment was 178 d and the Kaplan-Meier estimations of mortality among HIV-negative individuals were 4 and 5% at 6 mo for smear-positive and smear-negative TB, respectively. The corresponding mortality estimates for HIV-positive TB were 10.5 and 11% at 6 mo, respectively.

Prevalence Survey

Of the 762 participants in the community survey, 174 (23%) tested HIV positive, confirming the modeled HIV prevalence estimate. The survey sample also closely matched the predicted 2005 age, race, and sex distribution (data not shown). Among study participants, 11 individuals with TB were currently notified and receiving TB treatment. A further 12 previously undiagnosed definitive TB cases were identified: 6 smear-positive cases had either a second positive smear (n = 1) or culture of *M. tuberculosis* (n = 5); and 6 smear-negative cases had positive *M. tuberculosis* cultures obtained from two sputum specimens. Spoligotyping of DNA from these cultures demonstrated six distinct *M. tuberculosis* subtype patterns, with paired specimens from each individual showing identical patterns.

The HIV status of previously and newly identified cases of smear-negative and smear-positive TB is shown in Table 2. Of the 12 previously undiagnosed TB cases, 9 (75%) tested positive for HIV. Demographic characteristics of all participants with and without TB are shown in Table 3, together with symptoms and risk factors for TB. Prior imprisonment and HIV infection were significantly associated with notified TB, and HIV infection with previously undiagnosed TB. Cough, night sweats, loss of appetite, and weight loss were absent among 67% of those with previously undiagnosed TB. Two or more symptoms were present in 25% of previously undiagnosed TB cases compared with 22% among the no-TB group (p = 0.826).

Case-finding Proportions

In 2005, the point prevalence of adult PTB in the community (2,517/100,000; 95% CI, 2,225–2,837) was calculated by addition of notified case prevalence (notified incidence rate/median time on treatment = 942/100,000; 95% CI, 765–1146) and survey-determined untreated TB prevalence (1,575/100,000; 95% CI, 816–2,735). Based on this point prevalence, the proportion of cases identified and receiving TB treatment was only 37%. The prevalence of newly diagnosed smear-positive PTB among HIV-positive and -negative individuals was 2,837 of 100,000 and 175 of 100,000, respectively. Estimates of smear-positive PTB case-finding proportions for HIV-positive and -negative individuals, together with estimated time in the community before initiation of TB therapy, are given in Tables 4 and 5. The community

TABLE 2. PREVALENCE SURVEY OF 762 RANDOMLY SELECTED ADULTS

	Survey Sample (*n = 762*)*	HIV-positive Subjects (*n = 174*)	HIV-negative Subjects (*n = 588*)
Total pulmonary tuberculosis cases	23 (3.3)	16 (9.2)	7 (1.2)
Pulmonary tuberculosis cases on treatment	11 (1.4)	7 (4.0)	4 (0.7)
Newly identified			
Pulmonary tuberculosis	12 (1.6)	9 (5.2)	3 (0.5)
New direct sputum smear–positive cases	6 (0.8)	5 (2.9)	1 (0.2)
New culture positive			
New culture-positive smear-negative cases	6 (0.8)	4 (2.3)	2 (0.3)

Numbers (%) of identified treated cases of tuberculosis, previously undiagnosed pulmonary tuberculosis, previously undiagnosed direct sputum smear–positive and *Mycobacterium tuberculosis* culture-positive/smear-negative cases stratified by HIV infection status.

* Two patients refused HIV testing and two oral transudates were inadequate for testing. These four patients were all in the non-TB category.

TABLE 3. CHARACTERISTICS OF STUDY SAMPLE, OVERALL AND BY PREVALENT TUBERCULOSIS INFECTION, AMONG *762* PARTICIPANTS

	All Surveyed (*n = 762*)	No TB (*n = 739*)	Nonnotified TB (*n = 12*)	p Value	Notified TB (*n = 11*)	p Value
Demographics						
Median age, yr	27	27	26.5	0.81	30	0.12
Median school grade completed	11	11	11.5	0.31	8	0.06
Presently employed	398 (52)	390 (53)	7 (58)	0.78	1 (9)	0.004
Median household income, South African rands	1,300	1,300	1,400	0.98	578	0.009
Median residence, yr	5	5	5	0.83	6	0.33
Time away from home in last 6 mo	209 (27)	204 (28)	2 (17)	0.53	3 (27)	0.99
Median residents in household	3	3	3	0.07	3	0.85
Median persons sleeping in same room	2	2	2	0.81	2	0.65
Symptoms						
Cough	142 (19)	134 (18)	2 (17)	0.99	†	—
Night sweats	130 (17)	121 (16)	1 (8)	0.70	†	—
Loss of appetite	144 (19)	137 (19)	1 (8)	0.71	†	—
Loss of weight	207 (27)	193 (26)	4 (33)	0.52	†	—
Risk factors						
Ever had TB in past	61 (8)	58 (8)	0	0.61	3 (27)	0.053
Alcohol intake in past 6 mo	324 (43)	313 (42)	5 (42)	0.99	6 (55)	0.54
Visited shebeen (bar) in past 6 mo	180 (24)	175 (24)	3 (25)	0.99	2 (18)	0.99
Smoked in past 6 mo	205 (27)	196 (27)	4 (33)	0.53	5 (45)	0.18
Recreational drugs in past 6 mo	29 (4)	28 (4)	0	0.99	1 (9)	0.35
Employment history						
Past mining	43 (6)	41 (6)	1 (8)	0.50	1 (9)	0.47
Health care worker	21 (3)	20 (3)	0	0.99	1 (9)	0.27
Prison	11 (1)	8 (1)	1 (8)	0.14	2 (18)	0.008
HIV positive	174 (23)*	158 (22)	9 (75)	< 0.001	7 (64)	0.003

Definition of abbreviation: TB = tuberculosis.

All values are n (%) unless otherwise specified. p values are for comparing nonnotified TB (n = 12) and notified TB (n = 11) against no-TB (n = 739).

* Two patients refused HIV testing and two oral transudates were inadequate for testing. These four patients were all in the non-TB category.

† Symptoms questionnaire results do not represent prediagnosis symptoms and have been excluded.

burden of disease, estimated as the product of the number of affected individuals and the mean time before diagnosis and treatment, is presented in Figure 1. HIV-infected individuals were found to contribute 87% of the total person-years of undiagnosed disease.

DISCUSSION

This is the first population-based active case-finding survey of HIV and TB in sub-Saharan Africa. The major finding of this study was that, despite a well-run DOTS-based TB control program, 63% of community adult cases with PTB remained unrecognized to the TB treatment services. Among HIV-negative individuals, passive case finding identified 67% of prevalent smear-positive cases, which is close to the target of 70% suggested for adequate DOTS implementation (26). In contrast, among individuals with HIV infection, passive case finding only identified 33% of those with smear-positive TB. Thus, our study identified a huge unrecognized burden of TB in the community, predominantly among HIV-infected people. In this group, untreated smear-positive and smear-negative/culture-positive prevalence rates of TB were 2.9 and 2.3%, respectively. Among those with sputum smear–positive TB, the mean time spent in the community before accessing TB treatment was estimated to be as long for HIV-positive as for HIV-negative individuals, even after accounting for increased HIV mortality. HIV-infected individuals accounted for 87% of total person-years of untreated smear-positive TB before diagnosis and treatment. This finding is in contrast to an active case-finding study in South African

TABLE 4. PREVALENCE PER 100,000 OF NOTIFIED, TREATED ADULT CASES WITH PULMONARY TUBERCULOSIS AND UNTREATED CASES WITH PULMONARY TUBERCULOSIS IN THE COMMUNITY, WITH ESTIMATED CASE-FINDING PROPORTION AND TIME IN YEARS BEFORE INITIATION OF TREATMENT

	Total Population HIV Positive (*95% CI*)	Total Population HIV Negative (*95% CI*)
Prevalence of treated PTB	2,508 (1,924–3,210)	464 (327–639)
Prevalence of total (treated and untreated) PTB	7,648 (6,623–8,777)	978 (774–1,219)
Case-finding proportion*	0.34 (0.26–0.46)	0.48 (0.32–0.70)
Estimated mean time (yr) of TB patients before treatment†	1.19	1.02

Definition of abbreviations: CI = confidence interval: PTB = pulmonary tuberculosis.

* Prevalence of treated PTB/prevalence of treated and untreated PTB.

† Mean time untreated = PTB prevalence/(PTB incidence + HIV mortality rate), where the HIV mortality rate is the excess mortality associated with HIV infection in individuals notified with PTB 2002–2004.

TABLE 5. PREVALENCE PER 100,000 OF NOTIFIED, TREATED ADULT SPUTUM SMEAR–POSITIVE CASES WITH PULMONARY TUBERCULOSIS AND UNTREATED SPUTUM SMEAR–POSITIVE CASES WITH PULMONARY TUBERCULOSIS IN THE COMMUNITY, WITH ESTIMATED CASE-FINDING PROPORTION AND TIME IN YEARS BEFORE INITIATION OF TREATMENT

	HIV Positive, Smear Positive (*95% CI*)	HIV Negative, Smear Positive (*95% CI*)
Prevalence of treated PTB	1,563 (1,108–2,138)	352 (233–507)
Prevalence of total (treated and untreated) PTB	4,400 (3,619–5,299)	527 (380–711)
Case-finding proportion*	0.37 (025–0.53)	0.67 (0.41–1.0)
Estimated mean time (yr) of TB patients before treatment†	0.98	0.73

For definition of abbreviations, *see* Table 4.

* Prevalence of treated PTB/prevalence of treated and untreated PTB.

† Mean time untreated = PTB prevalence/(PTB incidence + HIV mortality rate), where the HIV mortality rate is the excess mortality associated with HIV infection in individuals notified with PTB 2002–2004.

gold miners, which estimated mean duration of smear-positive TB before diagnosis to be only 2 mo for HIV-positive miners (15). In that study, HIV-positive miners attended an HIV clinic and resulting ascertainment bias and use of isoniazid prophylaxis may have lowered the rate of previously undiagnosed smear-positive TB among the HIV-positive subjects (0.4%), resulting in a low calculated mean TB duration before diagnosis. The mean duration of infectiousness is a key determinant of the dynamics of the TB epidemic. In our study, the combination of high prevalence of untreated PTB among HIV-infected people, together with a prolonged exposure time in the community, may account for the lack of success of the DOTS program. However, TB infectivity may be lower for HIV-infected compared with HIV-uninfected individuals (27).

The study was performed in a community characterized by high HIV prevalence and increasing TB notification rates (20), and was sited within well-demarcated boundaries. There was a single community health clinic, making it likely that diagnosis and reporting of TB was uniform, thereby minimizing reporting biases. The TB DOTS control program performance over the 3 yr immediately before the survey demonstrated reasonable treatment completion rates but with a significant TB case fatality rate, which was higher in HIV-infected patients and those with smear-negative disease. The results of this survey reflect the epidemiology of TB and HIV in this community before access to highly active antiretroviral therapy (HAART) and without use of isoniazid prophylaxis.

The population-based survey identified individuals from whom *M. tuberculosis* was isolated but who did not perceive themselves to have symptoms of PTB. These individuals would therefore be less likely to present early to a TB control program. Our finding of a large burden of undiagnosed TB in this population is in contrast to a prior study in South Africa, which found a modest burden of undiagnosed TB in a rural population. However, in that study, laboratory investigation of TB was limited to only those with chronic cough and may therefore have underestimated the true TB burden (28).

Isolation of *M. tuberculosis* from asymptomatic individuals may initially appear to be in conflict with the international standards of TB care, which emphasize the investigation for TB among those with chronic cough (29). However, in a prospective population-based survey in the United States, symptoms of prolonged cough and fever were found to be insensitive predictors of TB (30). HIV infection has also been recognized to be a major modifier of the clinical presentation of TB, such that TB symptoms may be relatively minor (15, 16), atypical (11–13), or subclinical in HIV coinfected individuals (14). Furthermore, *post mortem* studies from Africa have reported TB as the cause of death in 38 to 47% of HIV-infected individuals in whom the diagnosis was suspected in only half while they were alive (31–33). In this poor community in our study, cough, night sweats, loss of appetite, and weight loss were reported frequently even in the absence of TB.

This study also highlights the problem of applying diagnostic tests developed primarily for clinic-based diagnosis to a population-based survey. Demonstration of acid-fast organisms on direct sputum smear lacks sensitivity (34) but has high specificity when sufficient organisms are present (35, 36). In contrast,

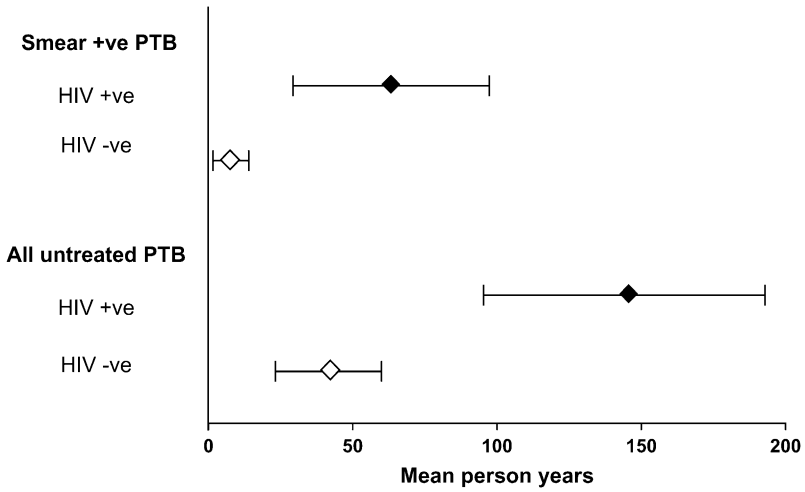

Figure 1. Community burden of undiagnosed pulmonary tuberculosis (PTB) by HIV and smear status. The *closed diamonds* (HIV +ve) and *open diamonds* (HIV −ve) indicate the estimated number of person-years, and the *horizontal lines* the 95% confidence intervals around these estimates. Estimated time before diagnosis is adjusted for increased mortality (13/100 patient-years) for HIV-positive individuals.

M. tuberculosis culture from sputum is sensitive but may be subject to false-positive results due to laboratory cross-contamination (37, 38). The performance characteristics of these diagnostic tests are of particular importance in population surveys. Limiting testing only to those with symptoms may increase the positive predictive value of TB tests; however, this strategy may miss a significant proportion of TB cases (30). In our study, restricting the case definition to those individuals with two positive laboratory results increased the specificity of diagnosis. Furthermore, false-positive cultures due to laboratory cross-contamination were very unlikely in our study because of the demonstration of multiple *M. tuberculosis* spoligotypes and 100% concordance among duplicate spoligotypes from each individual.

These data should be interpreted in light of several limitations. Although TB events are frequent in this population, the sample size was modest and therefore calculated prevalences have relatively wide confidence intervals.

There is evidence that many black African communities in this region have high degrees of mobility (39); however, time spent away from the community was not correlated with TB infection (either notified or nonnotified), suggesting that any such bias would have a minimal impact on the results. The response rate to the prevalence survey was not complete, although this response rate (78%) was relatively high and there were no differences in the distribution of age, sex, or race between our sample and the community based on population census data. Furthermore, the HIV prevalence observed in our sample (23%) was identical to modeling of the HIV epidemic in this setting (20). In addition, because our TB notification data come from routine service in which HIV testing is optional, HIV test results were available for only 81% of the notified TB cases; because we have assumed that the HIV prevalence among TB cases whose HIV status is unknown is similar to that of known individuals, these missing data are unlikely to affect our results substantially. Last, the generalizabilty of our findings will be dependent on the confirmation of our findings in other populations with very high HIV and TB burdens.

The results of this study have implications for individual management of HIV-infected individuals and the TB control program. Active screening of known HIV-infected individuals for TB, with induced sputum for sputum microscopy and culture, is indicated in this setting, even in the absence of symptoms suggestive of TB. Active screening for TB should precede initiation of isoniazid prophylaxis to avoid monotherapy of active TB, and screening based on symptoms may be inadequate. It will need to be prospectively evaluated whether screening for TB at entry to ART programs can identify the approximately 5% of individuals who develop TB soon after initiation of ART (40). TB control in this and similar communities will be dependent on the ability of the health system to identify HIV infections. Voluntary counseling and testing will need to be combined with subsequent active screening for TB of those who test HIV positive.

We have reported a large burden of HIV-associated undiagnosed TB in a community despite a well-functioning DOTS TB control program. Further population-based prevalence studies are required to confirm these findings in similar communities where HIV/TB coinfection is common, but our data indicate that such studies should not be limited to those with symptomatic disease. The passive case-finding aspect of the DOTS program performed inadequately for those with HIV infection and resulted in lower case-finding proportions in HIV-infected compared with HIV-uninfected individuals. The lack of symptoms suggestive of PTB may in part have contributed to this failure. Our data confirm that more intensive TB case finding in HIV-infected individuals is required, as has been previously suggested (21, 41–43).

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the manuscript.

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SUPPLEMENT ARTICLE

The Changing Natural History of Tuberculosis and HIV Coinfection in an Urban Area of Hyperendemicity

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Tuberculosis (TB) has proven to be difficult to control in regions with a high prevalence of human immunodeficiency virus (HIV) infection. We previously described high prevalence of HIV infection among adults (23%) and rapidly escalating TB notification rates in a peri-urban township, Site-M in Cape Town, South Africa. The combination of delineated boundaries, a well-characterized population, centralized TB record keeping, and high levels of HIV testing make this population uniquely suited for TB epidemiologic and transmission studies. The driver of the HIV and TB coepidemic appears to be a high annual risk of *Mycobacterium tuberculosis* infection in this community. A high annual risk of *M. tuberculosis* infection may be the result of unrecognized infections coupled with intense social interaction and crowding. New non–facility-based interventions will be required, with emphasis on community-based case finding and contact tracing to decrease the infective TB pool. There is a need for better understanding of the transmission dynamics of TB and the intensity of social interactions, which have exacerbated an HIV and TB epidemic in this community of hyperendemicity.

Tuberculosis (TB) remains a challenge to global public health, is a major cause of mortality, and has proven to be particularly difficult to control in regions with a high prevalence of human immunodeficiency virus (HIV) infection. An estimated 1.3 million deaths due to TB occur annually among HIV-uninfected individuals, and an additional 0.5 million deaths occur among HIV-infected persons. Of the estimated global burden of 9.3 million new TB cases in 2007, 1.37 million (14.8%) were associated with HIV infection and accounted for almost 25% of global AIDS-related mortality [1].

Sub-Saharan Africa has borne the brunt of the HIV and TB coepidemics, accounting for 79% of the global burden of HIV infection–associated TB cases in 2007. The 9 countries of the southern African region with hyperendemicity have TB case notification rates that are much higher than those for the rest of the African continent; these 9 countries have generalized HIV epidemics and report a prevalence of HIV infection of $\geqslant$50% among persons with newly diagnosed TB (Figure 1). In 2007, the estimated rate of TB case notifications in Africa was 161 cases per 100,000 population; however, in this subregion of hyperendemicity, incidence rates of TB in South Africa and Swaziland increased to 948 cases per 100,000 population and 1198 cases per 100,000 population, respectively, with 73% and 80% of new TB cases, respectively, involving HIV coinfection.

The Millennium Development Goals for global TB control are to halt and start to reverse the increasing incidence of TB and to halve the 1990 prevalence and death rates by 2015 [2]. In countries where TB is hyperendemic, such as South Africa and Swaziland, achievement of Millenium Development Goals for TB

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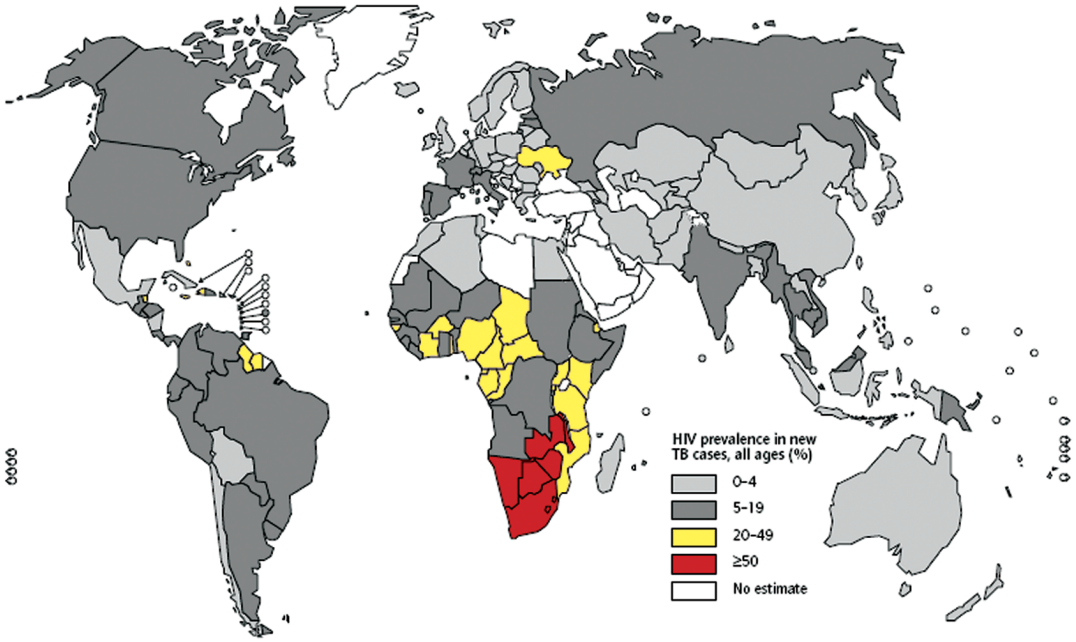

Figure 1. Estimated prevalence of HIV infection among persons with newly diagnosed cases of tuberculosis (TB), 2007. Reprinted with permission from the World Health Organization [1].

is unlikely, because it would require a reversal of present TB incidence trends and a 6-fold reduction in TB incidence during the next 6 years.

Since the World Health Organization (WHO) declaration in 1993 that TB was a global emergency, the directly observed therapy short-course (DOTS) strategy has been the key public health intervention that has been widely used to affect global TB control [3]. The strategy focuses on TB case management of sputum smear–positive cases with use of short-course rifampicin-containing chemotherapy. Case finding is passive and facility based, with emphasis placed on case retention and the achievement of a high cure rate. Although DOTS has been effective in most regions of the world, contributing to the sustained downward trend in global TB prevalence, it has been comparatively ineffective in countries with a high prevalence of HIV infection [1, 4–7]. During 2002–2004, the WHO and the Stop-TB Partnership published guidelines [8], a strategic framework [9], and an interim policy for TB and HIV infection [10] to address the specific challenge of HIV infection–associated TB. These interventions aim to reduce the burden of TB in HIV-infected persons through use of TB prevention strategies, including isoniazid preventive therapy (IPT), intensified case finding, and infection control in conjunction with antiretroviral therapy (ART)—the so-called "3 I's."

However, mathematical modeling suggests that a combination of very high levels of ART coverage and early ART initiation at high CD4 cell counts may be required to significantly affect population TB control, especially in settings where TB and HIV infection are hyperendemic [11]. Similarly, IPT is an intervention that reduces the risk of active TB in already HIV-infected individuals with latent TB infection rather than a primary strategy to control the public health burden of TB [12]. Although IPT is effective in decreasing the individual risk of progression to TB [13], the modeled population impact of IPT in areas of hyperendemicity is predicted to be small [14]. Therefore, there is an urgent need to understand the epidemiological factors driving the coepidemics in regions of hyperendemicity to inform TB-control strategies.

LESSONS FROM EPIDEMIOLOGIC STUDIES IN AN URBAN COMMUNITY WHERE TB IS HYPERENDEMIC

Although the global burden of HIV infection–associated TB is concentrated in the southern African subregion, there are large differences in disease burden even in the subregion. Specific subpopulations, such as South African mine workers, have been well documented to have a high TB incidence, in part because of the multiplicative effect of HIV infection and mine work–associated pulmonary silicosis [15]. In addition, rapid growth of urban areas is occurring in the context of generally declining economic performance, and the growth of urban areas includes huge numbers of persons with low-income status [16, 17]. It is estimated that ~61% of South Africans are urbanized, and

57% of these persons live in slum conditions [18] where TB and HIV burdens are greatest [19].

We previously described an epidemiologic study in South Africa that found a high prevalence of HIV infection among adults (23%) [5] and rapidly increasing TB notification rates [20]. Specifically, annual TB notifications have now reached 2000 cases per 100,000 population in this peri-urban township in Cape Town, designated by our study as "Site-M" [5, 20]. Regular household censuses have been performed that have shown that the community has undergone rapid population growth from 5000 residents in 1996 to 15,000 residents in 2008. This population growth has occurred within well-circumscribed boundaries (Figure 2). The community is socially deprived, living in overcrowded, largely informal dwellings located on demarcated plots serviced with water and sanitation. There is a single health care facility that provides primary medical care to community residents, and there is a primary and secondary school. Increases in TB notification rate have occurred despite a well-implemented national TB-control program based on the WHO DOTS strategy [21] at the single community clinic that manages all resident TB cases. Routine HIV testing (with consent) of patients with incident TB was introduced in 2002. The combination of delineated boundaries, a well-characterized population, centralized TB record keeping, and high levels of HIV testing make this population uniquely suited for studies on TB epidemiology and transmission.

IMPACT OF HIV INFECTION ON TB CONTROL

Almost 2 decades ago, before the development of effective combination ART, Styblo [6] reported that existing TB-control strategies would be significantly undermined by HIV infection, particularly in Africa. It was postulated that the impact of HIV infection on the epidemiological situation of TB would depend primarily on the following parameters: (1) the prevalence of HIV infection in a community, (2) the prevalence of TB in the

Figure 2. An aerial photograph of Site-M (in Cape Town, South Africa), with superimposed property boundaries outlined. The figure was created using ArcGIS, version 9.2 (ESRI, 380 New York St, Redlands, CA 92373-8100).

general population aged 15–49 years, (3) the progression from latent TB to active disease, (4) the level and trend in the annual risk of (new) TB, and (5) the detection rate of new and relapse cases of TB and cure rate among persons with smear-positive cases.

Other more recently recognized factors include the observation that combination ART can significantly decrease TB incidence [22] and the observation that TB incidence is very dependent on current CD4 cell counts [23]. Taking these factors into consideration, studies have focused on measuring the following likely drivers of increasing TB incidence in the study community: prevalence of HIV infection, prevalence of underlying latent TB, rates of progression from latent TB to active TB, annual risk of TB, and case detection rates.

PREVALENCE OF HIV INFECTION

Since 1990, the South African Department of Health has performed annual national surveys on the prevalence of HIV infection among women attending antenatal services [24]. The TB notification rates in South Africa from 1990 through 2005 [1] and the national antenatal seroprevalence of HIV infection are shown in Figure 3 [24]. The corresponding adult TB notification rates and prevalence of HIV infection among adults at Site-M from 1996 through 2005 are also shown in Figure 3. During the these periods, the seroprevalence of HIV infection increased markedly, reaching levels of 30% and 23% among national antenatal attendees and adults at Site-M, respectively. TB notification rates have increased logarithmically for linear increases in prevalence of HIV infection; an even stronger positive relationship was shown in the high-burden township. Possible explanations for this nonlinear relationship could include changes in CD4 cell count distribution in the HIV-infected population during the rapid-growth phase of the HIV epidemic or increasing TB transmission between HIV-infected individuals as the HIV epidemic grows rapidly.

AGE-SPECIFIC TB AND HIV INFECTION

Over the past decade the number of TB notifications has increased markedly at Site-M, with the increased burden of TB disease predominantly affecting persons aged 15–45 years (Figure 4*A*). The numbers of TB presentations at any age are a function of the number of individuals in each age strata and the TB rate specific to that age group. There have been significant changes to age-specific TB rates over the past decade that have been associated with increasing prevalence of HIV infection (Figure 4*B*). TB rates appear to have increased in all age groups; however, the most marked increases are among persons aged 15–44 years, the age group most at risk of acquisition of HIV infection.

POPULATION PREVALENCE OF TB

Prevalence of underlying latent TB at any age is influenced by both the prevailing TB transmission rate and transmission rates during the preceding years of life; therefore, prevalence of TB increases with increasing age because of accumulated exposure. The traditional way to measure latent TB is to measure reaction to tuberculin antigens by tuberculin skin testing. Population tuberculin skin testing surveys have been infrequently performed in recent decades; however, a recent tuberculin skin testing survey at Site-M township primary school reported TB prevalences that increased from 8% at school entry to 53% by

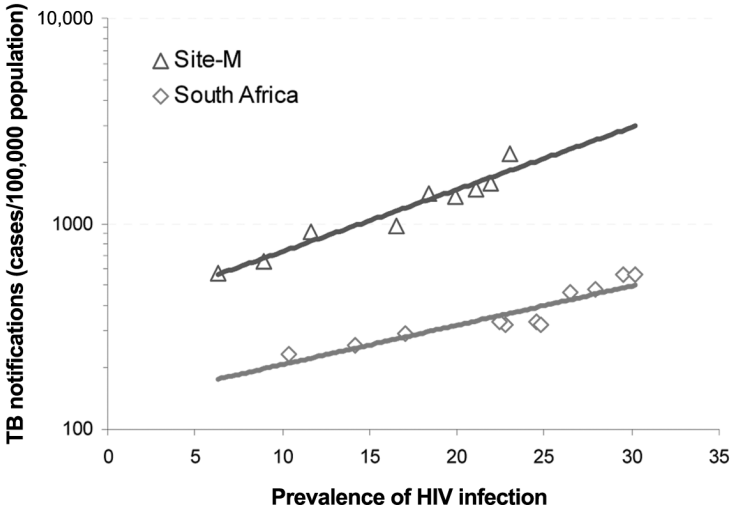

Figure 3. The relationship between tuberculosis (TB) notification rates and seroprevalence of HIV infection in the South African population *(diamonds)* and the population at Site-M *(triangles),* with exponential regression lines for South African data during 1990–2005 (R^2, 0.8461) and for Site-M data during 1996–2005 (R^2, 0.9376).

Figure 4. *A,* Number of tuberculosis (TB) notifications, stratified by age, at Site-M over two 4-year periods: 1996–1999 *(diamonds)* and 2004–2007 *(triangles). B,* TB notification rates, stratified by age, at Site-M over two 4-year periods: 1996–1999 *(diamonds)* and 2004–2007 *(triangles).*

the age of 15 years [25]. Moreover, prevalence of latent TB appeared to continue to increase throughout adolescence. In 2006, the HIV-uninfected control population at a similar nearby township in Cape Town had a TB prevalence of 77% by the age of 28 years [26]. Other similar township populations in Cape Town have also shown equally high prevalence of adult TB infection [27].

RATE OF PROGRESSION TO ACTIVE TB DISEASE

The temporal association between infection and risk of progression to active disease has been well recognized [28]. Progression to active disease is particularly rapid in children and has been a marker of ongoing transmission; however, the resultant TB disease is frequently sputum smear negative [29]. Childhood TB is conventionally reported internationally as a <15 years smear positive rate [1]. In 2007, South Africa reported a high smear positive childhood rate of 30 cases per 100,000 population. However, the high burden of childhood disease is not adequately reflected by the <15 years smear positive rate. In 2007, although the <15 year smear positive rate for Site-M was 81 cases per 100,000 population, the TB notification rate was 54 cases per 100,000 population among children <15 years of age and reached 1390 cases per 100,000 population among children <5 years of age.

There has been a marked change in the adult age of TB disease presentation. During 1996–1997, a period of relatively low prevalence of HIV infection, the incidence of TB increased progressively with advancing age, with no case notifications for adolescents (age, 10–19 years); however, TB notification rates increasing steadily to 1700 cases per 100,000 population in the fifth decade of life [5]. During 2003–2004, when the prevalence of HIV infection among adults exceeded 20%, TB notifications predominantly were for adolescents and young adults.

The estimated incidence of TB among HIV-uninfected and HIV-infected adult community members in 2005 was 953 cases per 100,000 population and 5140 cases per 100,000 population, respectively [20], indicating a 5-fold increased risk among HIV-infected individuals. As a consequence of these high TB incidence rates, the lifetime cumulative risk of TB is extremely high for both HIV-uninfected and HIV-infected individuals in this community. The very high cumulative lifetime TB risk for HIV-uninfected individuals is also much higher than the conventional estimated lifetime risk of latent infection progressing to TB disease of 10%–20% [30]. The increased lifetime risk may result from increased rates of progression because of poor nutrition, exogenous reinfection, or exposure to a high initial amount of infective TB [31]. The majority of individuals who are coinfected with TB and HIV live in sub-Saharan Africa, an area where hunger and malnutrition were already pressing concerns before the onset of the HIV and TB epidemics.

LEVEL AND TREND OF ANNUAL RISK OF TB

Population density varies markedly among and within countries. Both the nature of the dwelling and crowding within the dwelling will have an impact on the number of individuals exposed to an infected person. Site-M has had an increasing population density, reaching 15,700 persons/km^2 in 2008. The annual risk of TB among primary school children in 2008 was estimated to be 3.8%–4.8% [25], which is unprecedented in the current TB chemotherapeutic era. The annual risk of TB is similar to that found in several large scale surveys performed in western, eastern, and southern Africa from 1995 through 1960 [32]. In the prechemotherapy era, mean annual rates of infection as high as 13% per annum were reported among Parisian children in 1910 [33]. Lower rates of infection of 3% per annum were recorded among children in post–World War II Denmark [34].

A 77% prevalence of TB infection by age 28 years (during a period of increasing TB notifications) would indicate a high and ongoing mean risk of TB infection of 5.5% during an individual's preceding years of life. Childhood infection and TB disease in Site-M have been shown to be strongly associated

with exposure to adult smear-positive TB in combined family groups that are resident on each serviced plot [35].

In summary, the annual risk of TB in this community is extremely high and appears to be maintained or to increase throughout childhood and adolescence. Trends in the annual risk of TB over time in any specific age group in this community are less certain; however, there is little evidence for decreasing transmission.

CASE DETECTION AND TREATMENT

Efficient case management of infective TB is the cornerstone of the DOTS strategy [3], to which other supplementary control strategies may be added [8–10]. The single TB facility in Site-M implements DOTS-based, short-course, rifampicin-containing chemotherapy, administered in accordance with national guidelines [36]. TB-associated mortality during 2002–2004, before availability of ART, among HIV-infected and HIV-uninfected persons with TB was 13% and 3%, respectively [20]. Treatment completion rates of persons surviving to 6 months of age were 84% among HIV-infected persons and 86.6% among HIV-uninfected persons [20]. In 2005, a cross-sectional survey of a randomly selected subset of the general population found that the existing facility-based smear-positive case finding was higher for HIV-uninfected community members than for HIV-infected community members (rates, 0.67 [95% confidence interval, 0.25–0.53] and 0.37 [95% confidence interval, 0.41–1.0], respectively) [20].

TB TRANSMISSION PATTERNS

Over a 5-year period from 2001 through 2005, all acid-fast bacilli–positive sputum samples obtained at the single clinic in Site-M were cultured, and IS*6110*-based restriction fragment-length polymorphism analysis [37] was performed [38]. A broad diversity of ~200 distinct circulating *M. tuberculosis* strains were estimated to be circulating in this community—a finding consistent with other studies in sub-Saharan Africa [39, 40]. This study also found an association between W-Beijing family strains and HIV infection that may reflect ongoing transmission of TB among HIV-infected persons. W-Beijing strains have been associated with increased virulence [41] and the development of multidrug resistance [42]. The high degree of genotypic diversity in certain strains may indicate that they either are endemic in this population or may be emerging and diversifying in the community.

Another important finding was the high rate of strain clustering. In this study, approximately half of the strains were clustered, and there were close temporal associations, especially among the paired clusters, supporting the likelihood that a significant proportion of disease in the community is attributable to recent infections. No association was found between HIV infection and clustering; therefore, new infections may be occurring in both HIV-uninfected and HIV-infected patients.

DISCUSSION

This review has focused in detail on a specific, well-demarcated population that is heavily burdened with the dual epidemics of HIV infection and TB. Detailed analysis of the HIV and TB epidemics in this community may reveal insight into the factors driving the HIV and TB regional emergency in southern Africa. The incidence of TB has increased logarithmically with growth of the HIV epidemic and has been associated with a changed age distribution, resulting in the TB burden transferring from the elderly to young adults. The HIV epidemic appears to have unmasked a previously unrecognized high prevalence of TB and an unprecedented rate of TB in this crowded township.

Modeling studies suggest that a combination of interventions will be required to regain TB control [12]. The present strategy for global TB control remains the identification and effective case management of infectious TB cases [33]. However, although the facility-based program in this community appears to have achieved standard targets for TB case management for HIV-uninfected community members, the program has not had an impact on the extremely high annual risk of TB.

Strategies to decrease progression from prior infection may include ART and IPT. However, ART will need to be introduced with high coverage and earlier in the course of HIV infection, at higher CD4 cell counts, to significantly have an impact on rates of TB in the population [12]. Although the effect of ART is greater with continuing length of therapy [34], the benefits of IPT for HIV-infected patients are time-limited, compared with those for HIV-uninfected persons [14].

The underlying driver of the explosive HIV and TB coepidemic appears to be an extremely high annual risk of TB in this community. A high annual risk of TB may be the result of unrecognized infectious cases in the community and intense social interaction and crowding. New interventions in addition to the present clinic-based model of TB case management will be required, with increased emphasis on active community-based case finding and contact tracing to decrease the infective TB pool. There is also an urgent need for better understanding of the transmission dynamics of TB and intensity of social interactions that have exacerbated the HIV and TB coepidemic in this community of hyperendemicity.

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Antiretroviral Program Associated with Reduction in Untreated Prevalent Tuberculosis in a South African Township

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***Rationale*: In 2005, we reported high prevalence of untreated pulmonary tuberculosis (TB) in a South African community. Prevalent untreated TB is the main source of transmission. In settings with large burdens of human immunodeficiency virus (HIV) and TB, highly active antiretroviral therapy (HAART) may contribute to TB control.**

***Objectives*: To assess the community-level impact of HAART on TB prevalence, we repeated a community-based TB prevalence cross-sectional survey in 2008 following HAART roll-out.**

***Methods*: A random 10% adult population sample was identified from the community. Participants provided two sputum specimens for acid-fast bacilli microscopy and TB culture. Oral transudate specimen was collected for anonymous HIV testing, linked to TB diagnosis. An interviewer-administered, structured questionnaire identified TB and HIV history and risk factors.**

***Measurements and Main Results*: In the 2008 survey, 1,250 adults participated (90% response rate); 306 (25%) tested HIV positive, of which 60 (20%) were receiving HAART. A total of 20 TB cases were identified (12 receiving TB treatment), representing a significant decline in prevalence from 3.2 to 1.6% ($P = 0.02$) between the surveys. TB prevalence in participants not infected with HIV was unchanged ($P = 0.90$). The decline occurred among participants not infected with HIV, decreasing from 9.2 to 3.6% in 2005 to 2008, respectively ($P = 0.003$). In participants infected with HIV, prevalence of treated TB declined from 4 to 2.3% ($P = 0.06$), and untreated TB prevalence from 5.2 to 1.3% ($P = 0.02$). The proportion of untreated TB in patients receiving HAART decreased significantly, from 22 to 0% ($P < 0.001$).**

***Conclusions*: Prevalence of undiagnosed TB declined significantly over a period of increasing HAART availability. The decline was predominantly in individuals infected with HIV receiving HAART.**

Keywords: tuberculosis; prevalence; human immunodeficiency virus; antiretroviral therapy

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Prevalent untreated tuberculosis (TB) is the main source of *Mycobacterium tuberculosis*. However, little data are available on the impact of highly active antiretroviral therapy (HAART) on TB prevalence at a community level.

What This Study Adds to the Field

Our evidence suggests that the significant decline in undiagnosed TB prevalence is associated with the introduction of a rapid and large-scale HAART program, particularly in adults infected with human immunodeficiency virus. This decline is due to the increased case finding in the HAART program, and may also be a result of the impact of HAART-associated immune recovery on the risk of TB.

Tuberculosis (TB) prevalence is the proportion of a population with active TB disease, both treated (diagnosed) and untreated (undiagnosed), at a given point in time. Patients with TB receiving TB chemotherapy are considered to be less infectious (1), and it is this group of patients that is reflected in notification data. Untreated or inadequately treated TB disease, in contrast, is often unrecognized, and is the primary driver of transmission in a population (1–4). Therefore, prevalence of untreated TB is often considered a useful measure of this transmission factor.

One of the Stop TB Partnership targets is to reduce TB prevalence by 50% from 1990 to 2015 (5). The World Health Organization (WHO) has called for national surveys in high burden countries, such as South Africa, to monitor progress toward this target (5). However, evaluating population TB prevalence is logistically challenging, and cross-sectional surveys are costly due to the large sample sizes required, particularly in low-prevalence settings (6). Moreover, surveys require well trained staff and reliable laboratory services (6, 7), and, therefore, few population surveys have been performed in resource-poor settings (8–11). Reported surveys have often been limited to high-risk groups, such as prisoners (12–14), miners (15), or patients infected with human immunodeficiency virus (HIV) (16, 17). Thus, due to the paucity of national data, TB prevalence reported by the WHO for most countries is not measured directly, but is an indirect estimate based on related parameters (18). In the particular example of South Africa, prevalence data has been estimated from TB incidence and trends in TB mortality (5).

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In South Africa, 73% of TB incidence is HIV related (5). Highly active antiretroviral therapy (HAART) has been shown to reduce TB incidence in treatment cohorts (19, 20), and, therefore, wide-scale availability of HAART may play a role in TB control. However, little data are available on the impact of ART on TB prevalence—in particular, undiagnosed TB prevalence, at a community level.

In 2005, before the extensive roll-out of an ART program, we performed a community-based TB prevalence cross-sectional survey in a well defined, peri-urban township in South Africa. The survey reported a 3% overall TB prevalence in this community, and, in particular, a high rate of untreated, laboratory-confirmed pulmonary TB (PTB) among individuals infected with HIV (21). The residents of this community were of predominantly poor socioeconomic status, with high unemployment rates and overcrowded living conditions. The community had a high TB and HIV burden (21, 22), and was serviced by a single primary health care clinic. The clinic managed all patients with TB in accordance with the National TB Control Guidelines (23), which have not changed substantially since 2005. However, since 2005, there has been a significant scale-up of the ART program in this community, with ART coverage among individuals infected with HIV increasing from 5% in 2004 to 13% and 21% of the HIV-infected population in 2005 and 2008, respectively (24).

To assess the impact of a wide-scale HAART program on TB prevalence in this community, we repeated the cross-sectional survey in 2008, measuring both TB disease and HIV infection. Some of the results of these studies have been previously reported in the form of a conference abstract (25).

METHODS

This study was performed from June to December 2008, and the same methodology as the 2005 survey was used (21). A house-to-house enumeration of the community provided a database of 14,592 residents, of whom 1,500 residents 15 years of age or older were randomly selected for study participation (10% of the community).

All participants completed a structured questionnaire investigating participant demographic characteristics, TB history, TB symptoms (cough, night sweats, loss of appetite, and loss of weight), risk factors for TB (including housing, alcohol use, smoking, recreational drug use, prior incarceration, and employment history), and risk factors for HIV (including number of sexual partners and condom use). Questionnaires were interviewer administered in the participants' home language. Two sputum specimens were collected from each participant: an early-morning sputum produced at home, and a second, induced sputum collected at the site with saline nebulization. Both sputum specimens were tested at the same laboratory for acid-fast bacilli (AFB) by microscopy and for *Mycobacterium tuberculosis* (*Mtb*) growth by culture. An oral transudate specimen was collected for anonymous HIV testing, with HIV results linked to TB diagnosis. The study was approved by the Research Ethics Committee of the University of Cape Town, and all participants provided written informed consent.

Laboratory Procedures

Sputum specimen smears were examined for AFBs with an auramine-O stain. Sputum sediments were cultured in the mycobacterial growth indicator tubes automated system (Sparks, MD) and incubated for 6–8 weeks before being reported as negative. Positive cultures were examined for the presence of AFB by Ziehl Neelsen staining, and were identified as *Mtb complex* with a polymerase chain reaction assay. The oral mucosal transudate specimen for HIV testing was collected with the Orasure oral fluid collection device (Orasure Technologies, Bethlehem, PA). The Vironostika Uni-Form II HIV-1 and HIV-2 plus 0 ELISA test (bioMérieux SA, Marcy l'Etoile, France) was used to test for HIV-1 and HIV-2 antibodies.

Case Definitions

Following on the 2005 methodology, participants who reported on the questionnaire that they were currently receiving TB treatment were classified as "treated TB cases." "Untreated TB cases" were defined as participants' without a prior known TB diagnosis, but with laboratory-confirmed infection, as defined by two positive AFB smear results or two positive *Mtb* culture results, or a positive AFB smear result confirmed by a positive *Mtb* culture on separate specimens. All untreated TB cases were referred to the local TB clinic for chemotherapy.

Statistical Analysis

Data were analyzed with STATA 10.0 (StataCorp, College Station, TX).

Bivariate analyses employed Student's *t*, Wilcoxon's rank sum, and Chi-square tests, as appropriate. Multiple logistic regression models were developed to examine changes in overall TB prevalence, as well as treated and untreated TB prevalence between the two surveys, after adjusting for variation in individual participant characteristics. These models were weighted for the proportion of the population sampled in each survey. Median CD4 counts were calculated for the total HAART cohorts in 2005 and 2008, based on each HAART patients' averaged CD4 count recorded in the survey year. Annual median CD4 counts were compared across the two years with Wilcoxon's rank sum test. Case-finding proportion was calculated as the proportion of prevalent cases, overall and by HIV strata, that were reported as treated TB cases. The 95% confidence intervals (CIs) were based on the Poisson distribution, and all statistical tests were two-sided (at $\alpha = 0.05$).

RESULTS

Of the 1,500 residents selected for participation in the 2008 survey, home visits confirmed that 1,383 of these individuals were still resident in the community, and eligible for the study. Of these, 1,250 (90%) consented to enroll in the study, 121 (9%) refused participation and 12 (1%) were not contacted after five home visits. In the initial survey (2005), 762 (78%) of 971 randomly selected, eligible residents were enrolled in the study, with a refusal rate of 15% (21). Demographic characteristics of the two samples are shown in Table 1.

TABLE 1. CHARACTERISTICS OF TWO SURVEY SAMPLES

Characteristics	2005 Survey	2008 Survey
Community size (≥15 yr old), *n*	9,935	11,958
Study sample, *n*	762 (15% refusal)	1,250 (9% refusal)
Demographics		
Median age (IQR), yr	27 (22–35)	27 (22–33)
Male sex, *n* (%)	340 (45)	648 (52)
Median school grade completed (IQR)	10 (8–11)	10 (8–11)
Presently employed, *n* (%)	398 (52)	662 (53)
Median residence, yr (IQR)	5 (3–7)	5 (2–10)
Median residents in household (IQR)	3 (2–5)	3 (2–4)
Median persons sleeping in same Room (IQR)	2 (2–3)	2 (1–3)
Risk factors, *n* (%)		
Ever had TB in past	58 (8)	101 (8)
Alcohol intake in past 6 mo	324 (43)	408 (33)
Visited shebeen (bar) in past 6 mo	180 (24)	261 (21)
Smoked in past 6 mo	205 (27)	289 (23)
Recreational drugs in past 6 mo	29 (4)	54 (4)
Employment History, *n* (%)		
Past mining	43 (6)	44 (4)
Health Care Worker	21 (3)	21 (2)
Prison in the past 6 mo	11 (1)	15 (1)
HIV positive (95% CI)*	174 (23; 20–26%)	306 (25; 22–27%)
HIV positive on HAART (% of HIV infected; 95% CI)	9 (5; 2–10%)	60 (20; 15–25%)

Definition of abbreviations: CI = confidence interval; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; IQR = Interquartile range; TB = tuberculosis.

* In 2005, four participants declined HIV testing, and in 2008, 43 participants declined HIV testing.

TABLE 2. TUBERCULOSIS PREVALENCE SURVEY RESULTS, OVERALL AND BY HUMAN IMMUNODEFICIENCY VIRUS AND ANTIRETROVIRAL THERAPY STATUS, 2005 AND 2008

Tuberculosis Survey	No. at Risk	Total Prevalence % (*n*)	Treated Prevalence % (*n*)	Untreated Prevalence % (*n*)	Case-Finding Proportion (%)
2005					
Total TB cases	762	3.0 (23)	1.5 (11)	1.6 (12)	48
HIV-negative cases	584	1.2 (7)	0.7 (4)	0.5 (3)	57
HIV-positive cases	174	9.2 (16)	4.0 (7)	5.2 (9)	44
HIV-positive no ART	165	7.3 (12)	3.0 (5)	4.2 (7)	42
HIV-positive ART	9	44.4 (4)	22.2 (2)	22.2 (2)	50
2008					
Total TB cases	1250	1.6 (20)	1.0 (12)	0.6 (8)	60
HIV-negative cases	901	1.0 (9)	0.6 (5)	0.4 (4)	56
HIV-positive cases	306	3.6 (11)	2.3 (7)	1.3 (4)	64
HIV-positive no ART	246	2.8 (7)	1.2 (3)	1.6 (4)	43
HIV-positive ART	60	6.7 (4)	6.7 (4)	0.0 (0)	100

Definition of abbreviations: ART = antiretroviral therapy; HIV = human immunodeficiency virus; TB = tuberculosis.

In 2005, overall, 23% (95% CI, 20–26%) of the participants were HIV infected, and four participants (1%) declined HIV testing (21). Of the 174 participants infected with HIV, 5% (95% CI, 2–10%) were receiving HAART. In 2008, 43 participants (3%) declined HIV testing; among those that were tested, 25% (95% CI, 22–27%) were HIV infected ($P = 0.23$), and, of these, 20% (95% CI, 14–23%) were receiving HAART.

Total Study Population TB prevalence

In 2008, 12 participants (1%) reported receiving TB treatment at the time of study participation, and a further eight (0.6%) untreated cases were identified. The prevalence results of the 2005 and 2008 surveys, overall and by HIV strata, are reported in Table 2 and shown in Figure 1. The overall TB prevalence (treated and untreated cases) declined significantly, from 3% in 2005 (21) to 1.6% in 2008 ($P = 0.04$). When adjusted for age, sex, HIV status, as well as demographic and risk profile characteristics that differed between the two surveys, this reduction in prevalence remained significant ($P = 0.05$), as shown in Table 3. The reduction in prevalent treated TB (from 1.5 to 1.0%) was not significant (adjusted $P = 0.34$); however, the reduction in prevalent untreated TB from 1.6 to 0.6% was significant, and remained significant after adjustment as described previously here (crude and adjusted $P = 0.05$) (Table 3).

In 2005, 50% ($n = 6$) of the untreated TB cases had smear-positive PTB (21), whereas, in 2008, 13% ($n = 1$) of the untreated cases had smear-positive PTB ($P = 0.09$).

In 2008, untreated TB was not associated with reported cough ($P = 0.90$), night sweats ($P = 0.35$), loss of appetite ($P = 0.32$), or loss of weight ($P = 0.22$). This was in keeping with the 2005 survey findings (21).

Participants Not Infected with HIV

The total TB prevalence in participants not infected with HIV remained unchanged between surveys (adjusted $P = 0.90$), as did the prevalence of both treated TB (adjusted $P = 0.90$) and untreated TB (adjusted $P = 0.69$) in this group.

Participants Infected with HIV

The total TB prevalence dropped significantly in participants infected with HIV, from 9.2 to 3.6% (adjusted $P = 0.013$). Although the decrease in treated TB prevalence was not significant in this group (4.0% in 2005 versus 2.3% in 2008; $P = 0.22$ after adjustment for age, sex, HAART status, and demographic and risk profile characteristics, as described previously here), the prevalence of untreated TB cases declined significantly, from 5.2% in 2005 to 1.3% in 2008 (adjusted $P = 0.02$). The multivariate logistic models for total TB prevalence and untreated TB prevalence in participants infected with HIV are reported in Table 4.

HAART

The distribution of treated and untreated TB cases by HAART status in patients infected with HIV is shown in Table 2 (2005

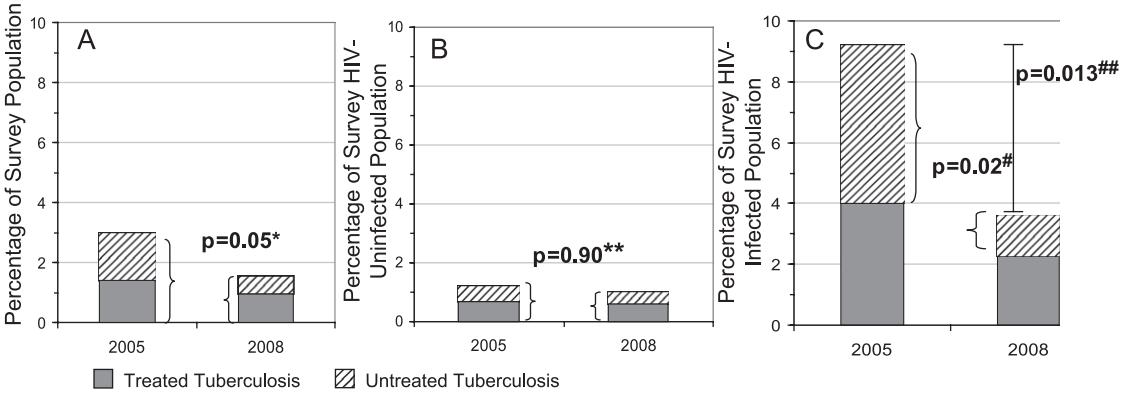

Figure 1. Prevalence of treated and untreated tuberculosis (TB) in the (*A*) total study sample, and in (*B*) human immunodeficiency virus (HIV)–uninfected and (*C*) participants infected with HIV. *Adjusted for age, sex, HIV status, education level, mean residents in household, reported alcohol use, smoking, and history of working in a mine; **adjusted for age, sex, education level, mean residents in household, reported alcohol use, smoking, and history of working in a mine; †comparison of untreated TB in HIV-infected population, adjusted for age, sex, education level, mean residents in household, reported alcohol use, smoking, and history of working in a mine; ††comparison of overall TB in HIV-infected population, adjusted for age, sex, education level, mean residents in household, reported alcohol use, smoking, and history of working in a mine.

TABLE 3. MULTIVARIATE LOGISTIC MODEL FOR OVERALL, TREATED, AND UNTREATED TUBERCULOSIS PREVALENCE IN THE TOTAL STUDY POPULATION

	Total TB Prevalence		Treated TB Prevalence		Untreated TB Prevalence	
	OR (*95% CI*)	*P* Value	OR (*95% CI*)	*P* Value	OR (*95% CI*)	*P* Value
Survey year						
2005	1		1		1	
2008	0.53 (0.28–0.97)	0.05	0.66 (0.28–1.56)	0.34	0.39 (0.15–0.96)	0.05
Age, yr	1.01 (0.97–1.05)	0.61	1.03 (0.97–1.07)	0.19	0.98 (0.91–1.04)	0.48
Sex						
Male	1		1		1	
Female	0.88 (0.40–1.96)	0.75	0.71 (0.25–2.09)	0.52	1.08 (0.31–3.84)	0.90
Education, yr in school	0.99 (0.89–1.11)	0.92	0.90 (0.79–1.02)	0.11	1.26 (0.99–1.60)	0.06
No. of residents in household	1.08 (0.94–1.25)	0.29	1.17 (0.98–1.40)	0.09	1.00 (0.78–1.27)	0.98
Alcohol intake in past 6 mo	0.96 (0.46–1.97)	0.90	0.84 (0.31–2.31)	0.74	1.12 (0.40–3.12)	0.83
Smoked in past 6 mo	2.14 (0.94–4.90)	0.07	2.27 (0.74–6.90)	0.15	1.77 (0.49–6.40)	0.38
Past employment in mines	1.97 (0.62–6.30)	0.25	0.34 (0.04–3.03)	0.34	12.19 (2.48–59.92)	0.002
HIV status						
HIV negative	1		1		1	
HIV positive	6.42 (3.34–12.32)	<0.001	6.14 (2.55–14.80)	<0.001	6.48 (2.43–17.25)	<0.001

Definition of abbreviations: CI = confidence interval; HIV = human immunodeficiency virus; OR = odds ratio; TB = tuberculosis.

and 2008). The proportion of overall and untreated TB cases on HAART decreased significantly from 2005 to 2008 ($P = 0.01$ and $P < 0.001$, respectively). The median CD4 count for the total HAART cohort in this community was 269 (interquartile range, 177–350) in 2005, and 350 (interquartile range, 240–504) in 2008 ($P < 0.001$).

Case-Finding Proportions

As shown in Table 2, the case-finding proportion by the TB clinic in this community increased from 48% in 2005 to 60% in 2008. Case-finding proportions did not change significantly in participants not infected with HIV (57 versus 56%). However, in the HIV-infected population, case finding increased substantially, from 44 to 64%. Case finding increased for patients on HAART, from 50% in 2005 to 100% in 2008.

DISCUSSION

To our knowledge, this is the first repeated cross-sectional prevalence survey following the large-scale availability of HAART in a sub-Saharan Africa community. The study was performed among randomly selected individuals in a well defined community, with HIV and HAART data linked to TB results. The main finding was that the prevalence of PTB in adults declined significantly between 2005 and 2008, and this decline was due to a nearly threefold decrease in TB prevalence in the HIV-infected population(from 9.2 to 3.6%). In the HIV-infected group, the decrease was predominantly due to a fourfold decline in untreated TB. In patients infected with HIV on HAART, TB prevalence dropped from 44 to 6.7%, and the largest decline was also seen in untreated TB cases.

To explain these findings, we postulate that a widespread HAART program can decrease prevalent TB in a community through two mechanisms: increased TB active case finding, and immune recovery associated with HAART.

Increased Case Finding in the HAART program

When patients entered the HAART program, they underwent active screening for TB, based on the National and WHO policy (26, 27). Screening for TB is based on symptom review. In patients with symptoms suggestive of TB, sputum staining for AFB and/or culture for *Mtb* growth is performed. The implementation of this policy is demonstrated by the increased proportion of case finding in the HIV-infected population, from 2005 to 2008, whereas the case-finding proportion in the HIV-uninfected population remained unchanged. This has resulted in a significant decrease in the previously large burden of undiagnosed, untreated TB in patients infected with HIV reported in the first survey. Furthermore, the decrease in untreated, smear-positive TB cases was greater than the decrease in the

TABLE 4. MULTIVARIATE LOGISTIC MODEL FOR OVERALL AND UNTREATED TUBERCULOSIS PREVALENCE IN THE HUMAN IMMUNODEFICIENCY VIRUS–INFECTED STUDY POPULATION

	Total TB prevalence OR (*95% CI*)	*P* Value	Untreated TB prevalence OR (*95% CI*)	*P* Value
Survey year				
2005	1		1	
2008	0.35 (0.15–0.80)	0.01	0.20 (0.07–0.74)	0.02
Age (in years)	1.03 (0.98–1.09)	0.22	1.04 (0.96–1.14)	0.29
Sex				
Male	1		1	
Female	0.93 (0.31–2.83)	0.90	1.40 (0.23–8.45)	0.72
Education, yr in school	1.00 (0.87–1.16)	0.95	1.24 (0.92–1.67)	0.15
No. of residents in household	0.98 (0.79–1.23)	0.86	0.80 (0.55–1.17)	0.25
Alcohol intake in past 6 mo	1.01 (0.41–2.50)	0.99	1.80 (0.50–6.43)	0.37
Smoked in past 6 mo	1.26 (0.40–3.98)	0.70	1.57 (0.28–8.97)	0.61
Past employment in mines	2.81 (0.67–11.76)	0.16	7.44 (1.12–49.58)	0.04

Definition of abbreviations: CI = confidence interval; OR = odds ratio; TB = tuberculosis.

overall untreated TB prevalence, and this finding may suggest that active case finding is removing the more infectious cases (28, 29) from the community. It is therefore possible that part of the decrease in TB prevalence may be due to a reduction in TB transmission. Patients infected with HIV have a more rapid rate of progression to TB disease after recent infection compared with individuals not infected with HIV (30). As a result, any benefit to the HIV-uninfected population accruing from decreased transmission may not be evident in the short interval between these surveys. The impact of active-case finding on TB prevalence is in keeping with results of mathematical models, which have assessed the impact of intervention strategies on population TB rates (31, 32).

Risk of Disease: Immune Recovery on HAART

Active case finding would transfer untreated TB cases into the treated, notified group. However, rates of treated TB did not increase in this population; in contrast, treated TB in participants infected with HIV had declined between the two surveys. This finding may reflect changes in the immune status of the HIV-infected population related to high HAART coverage. It is well documented that HAART, and the subsequent CD4 count recovery, is associated with a substantial reduction in TB risk in patients infected with HIV (19, 33–35). The rapid scale-up of the ARV program in this community (with 20% of the HIV-infected population receiving HAART in 2008) would have resulted in a large treatment cohort, with an increasing mean CD4 count (as found in treatment cohorts in similar settings [36]), and, therefore, a decrease in risk of TB disease in patients infected with HIV. Although the ART cohort had a higher mean CD4 count in 2008 compared with 2005, the median CD4 count in 2008 was still relatively low. Therefore, it is possible that the impact on reduction of TB prevalence will continue to increase with ongoing ART initiation and accumulative immune recovery.

Alternative Explanations for Study Findings

The decrease in TB prevalence in this community is unlikely to be due to changes in the TB control program other than the active case finding in HAART patients, nor to increased population awareness of TB due to the surveys. This is supported by the stable rates of treated and untreated TB in participants who are HIV negative in this study, and furthermore, that overall TB notification rates of participants who are HIV negative have remained constant over the last decade (37). Similarly, TB-associated mortality rates have declined in this community (data not shown), particularly in patients infected with HIV, and, as such, an increase in mortality is not likely to be responsible for the decrease in TB prevalence. Emigration could result in a decreased prevalence of disease, but biennial censuses, performed from 2002 to 2008, show that net immigration is greater than emigration in this growing community, with a 12% increase in population size between the two surveys. Furthermore, the same TB control program laboratory services, with consistent protocols, were used for the microscopy, culture, and differentiation of sputum specimens in both surveys.

It should be noted that the 2008 survey participants had a lower TB risk profile compared with the participants in the 2005 survey. However, after adjusting for these factors, the decrease in both overall TB prevalence as well as untreated TB prevalence in participants infected with HIV between the two surveys remained statistically significant. HIV testing was accepted by 98% of the participants (averaged across both surveys); however, HIV status was available for all TB cases, both treated and untreated. This relatively small community is typical of many recently urbanized populations in South Africa, but there is a need for further investigation to confirm our findings in other high HIV and TB prevalence settings.

Undiagnosed TB prevalence is an important driver of TB transmission (1–4). We have shown a significant decline in adult TB prevalence—in particular, undiagnosed TB prevalence in patients infected with HIV—associated with the introduction of a rapid and large-scale HAART program in this community. This decline appears to be due to the increased case finding in the HAART program, and may also be a result of the impact of HAART-associated immune recovery on TB risk. These findings suggest that large-scale HAART programs may contribute to TB control in high HIV prevalence settings.

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Tuberculosis control has failed in South Africa – time to reappraise strategy

Robin Wood, Stephen D Lawn, Simon Johnstone-Robertson, Linda-Gail Bekker

South Africa's rate of tuberculosis (TB) has increased over the last 20 years, to now having the third-highest TB burden in the world. The TB control programme has primarily focused on effective case management of passively presenting TB cases, and progress has been recorded towards international treatment targets. While outcomes for notified TB cases have improved, this strategy failed to contain the TB epidemic. South Africa has the highest per capita annual risk of TB disease of comparably sized countries globally, and its communities have extremely high TB transmission rates. The rates of TB infection of children and adolescents are now similar to those reported 100 years ago in Europe long before chemotherapy became available. High rates of HIV testing of TB patients in Cape Town allows analysis of TB notification data stratified by age, type of TB and HIV status, and a better understanding of TB epidemiology. TB infection prevalence data from Cape Town communities allow estimation of the prevailing force of TB infection and, together with TB notification and prevalence data, the effective number of secondary infections and case finding proportions can be estimated. This better understanding of the major drivers of the TB epidemic allows reasons to be identified for failure of the present strategy. New control strategies can also be identified, that must be accompanied by novel TB control targets.

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South African reports to the World Health Organization (WHO) indicate that its tuberculosis (TB) notifications have increased fivefold over the last 20 years; in 2008, South Africa (SA) had the third-highest TB burden, after India and China.[1] SA and Swaziland now have the highest TB notification rates in the world, with about 1% of their populations developing TB annually.[1,2] SA was responsible for approximately 25% of the global burden of HIV-associated TB cases in 2007.[2]

While a worsening epidemic is revealed, data give little insight into understanding why TB control is failing. To better understand the epidemiology of TB control, the national and available community and city level data must be integrated; understanding this provides insights into the weaknesses of existing strategies and permits the development of additional rational interventions to regain TB control.

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TB notifications

In 2007, 315 000 cases of TB were notified in South Africa – a rate of new and recurrent disease of 649 per 100 000 population.[2] Approximately 40% of nationally notified cases were tested for HIV infection, of which 73% were estimated to be positive, but this high prevalence may be affected by selection bias. The 2009 Cape Town (population 3.4 million) notification of 31 095 TB cases[3] represents double the number of TB cases reported in the USA (population >300 million people).[2]

Provider-initiated HIV testing in Cape Town TB clinics has increased to over 85%, allowing analysis of data stratified by age and HIV status, and providing a better understanding of the epidemiology of TB in a large urban population. Using denominators from the Cape Town population structure (Calle Hedberg, City of Cape Town and Stats SA) and TB notification data, age-specific TB notification rates stratified by HIV status were derived (Fig. 1). HIV-associated TB accounted for 44% of the total case burden. The peak TB notification

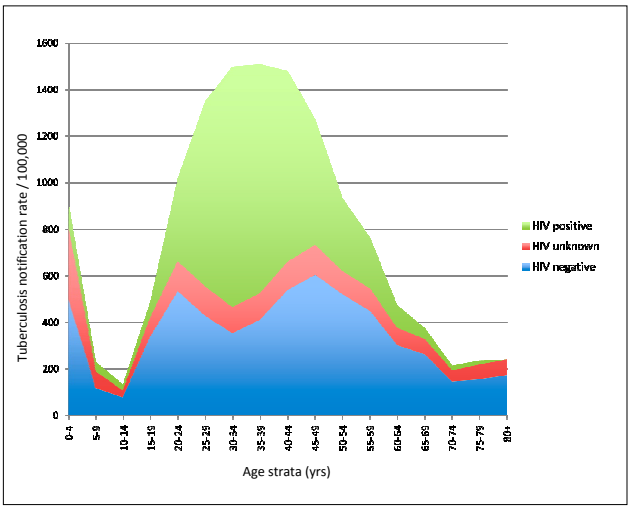

Fig. 1. TB notifications in 2009 for the City of Cape Town stratified by 5-year age groups and by provider-initiated HIV testing results. The demominators for age strata derived from National Department of Health/Health Information System Programme by disaggregating StatsSA district estimates (November 2009) using data from the `Small Area Layer` (StatsSA, 2004).

rate was among young adults, exceeding 1 400 per 100 000 population (Fig. 1). Of those aged between 25 years and 45 years, 63% of TB cases were HIV-associated. Of particular concern is that about 1% of children <5 years old were notified as having TB. Since children rapidly progress from TB infection to TB disease, childhood TB disease indicates recent ongoing TB transmission.[4,5] These data strongly indicate very high transmission rates in the community.

The cumulative lifetime risk of new and recurrent TB of HIV-uninfected individuals living in Cape Town was calculated by cumulatively adding the annual incidence of each 5-year age grouping (Fig. 2). The denominators for these calculations are the total age strata populations. As the HIV-infected population is included in the denominator, the resultant rate estimations are conservative. With the *status quo*, 1 in 5 individuals resident in Cape Town who remain HIV-uninfected will be at risk for developing TB before reaching the age of 60 years. This modern lifetime risk is unprecedented and is approximately twice that for individuals acquiring TB infection in the UK in the 1950s.[6] The cumulative risk of TB disease for those who become HIV-infected is considerably higher. Therefore our TB control strategies have failed, resulting in the highest reported global rates of TB disease in children and HIV-infected and uninfected adults.

TB infection

The epidemiological transitions relevant to the pathogenesis of TB from exposure to death or cure are outlined in Fig. 3. As with other infectious diseases, the key primary event is the initial acquisition of infection. While there have been no recent systematic attempts to measure TB infection rates in South Africa, data from Cape Town report extremely high prevalence rates of TB infection among children and adolescents.[7-10]

In 2005, the prevalence of a positive (>10 mm induration) tuberculin skin test (TST) among 7 457 primary school children (median age 8.6 years) was 37.4%.[7] A TST survey in a Cape Town school in 2005 reported TB prevalence to be 26.2% in 5 - 8-year-olds, increasing to 52.5% in 14 - 17-year-olds.[8] The prevalence of TB infection increased from 20% at school entry to 52% at 15 years, and reached 75% at 25 years in another study including HIV-negative adolescents and adults.[9] Note also that a further 8% of the population will have developed TB disease by the age of 25 years (Fig. 2) and they were excluded from the prevalence surveys. In 2 neighboring urban communities with low HIV prevalence, a high prevalence of TB infection in children (6 - 9 years) was reported.[10] Transmission rates

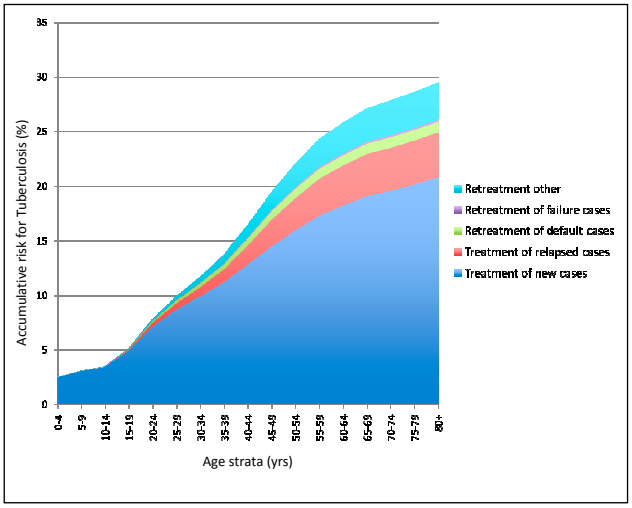

Fig. 2. The accumulated life-time risk of being notified with new or relapsed TB calculated for HIV-negative individuals. Values are based on cumulative 2009 age-specific TB notification rates for Cape Town.

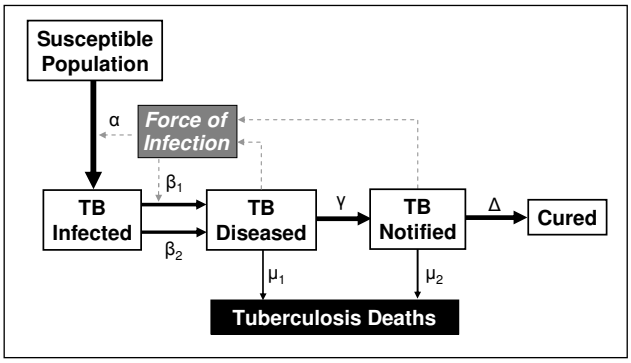

Fig. 3. An outline of TB transition states for a susceptible population from initial infection to disease and treatment outcome or death. The force of infection is shown as a function of prevalent untreated TB disease and determines the rate of primary TB infection (α) and the rate of multiple infection exposures (β_1) of latently infected individuals. β_2 is the progression rate from latent to active disease, γ the case-finding proportion, Δ the cure rate, and μ_1 and μ_2 the off- and on-chemotherapy mortality rates respectively.

increased between 1998 and 2005 and remained among the highest (4.1 - 5.8% per annum) in the world.[10]

Collectively, these studies indicate an extremely high prevalence of TB infection in Cape Town acquired during childhood, and that high rates of acquisition of TB infection continue throughout adolescence and into young adulthood. These high rates of TB transmission predate the HIV epidemic and have not declined over the last decade. Together with increasing TB notification rates, these data clearly indicate a failure of TB control.

Force of TB infection

The force of infection is the proportion of TB-uninfected individuals newly infected per annum, and is principally determined by the prevalence of infectious TB cases and the effective number of secondary cases infected by each infectious case. A TB prevalence survey of a random sample of the general population was conducted in a Cape township in 2005. The prevalence of laboratory-proven TB among HIV-uninfected and infected adults was 0.47% and 5.2% respectively.[11] After the community scale-up of antiretroviral therapy (ART), the prevalence of TB among the HIV-uninfected population did not change, but the prevalence among HIV-infected adults decreased from 5.2% to 1.3%.[12]

Cape Town studies estimated the force of TB infection to be between 4% and 8% per annum.[7-10] In historical perspective, these values are similar to the force of infection estimated for the UK population in the early decades of the 20th century, which was long before TB chemotherapy was developed. In 1900 in the UK, the force of infection was 10% per annum, declining to 1% around 1950 and to <0.01% by 2000.[13]

Fig. 4 shows the relationship between increasing force of infection and the proportion of the population with primary TB infection and the proportion multiply exposed to TB infection. Effective TB control interventions that produced small reductions in the force of infection in Cape Town could produce large benefits in transmission reduction. Reduction of the force of infection to 1% would decrease the proportion of the population exposed to multiple TB exposures from over 50% to <0.5% (β_1 in Fig. 3) and the proportion acquiring primary infection would decrease from 75% to <30% (α in Fig. 3).

Effective contact number

An effective TB contact is defined as a contact between an infectious pulmonary case and a susceptible individual, sufficient to result in TB infection.[14] The number of individuals infected by each case (the

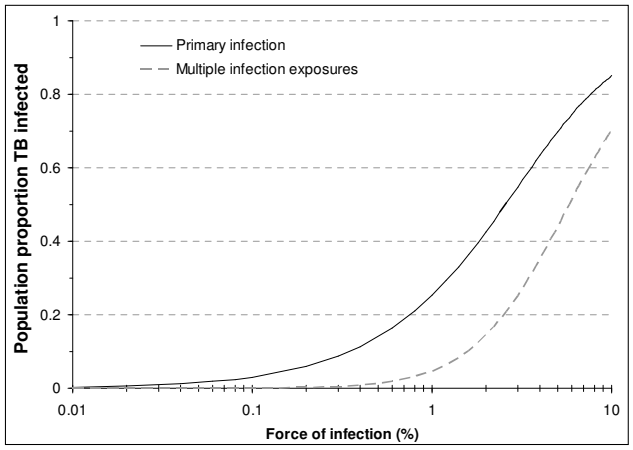

Fig. 4. The relationship between 'force of tuberculosis infection' and proportion of population acquiring primary TB infection and multiple infectious exposures. The prevalence of primary infection is defined as $P_1=[^{60}\Sigma_{x=0}(1-\exp(-Fx)]/61$ *and the prevalence of multiple infection exposures is defined as* $P_2=[^{60}\Sigma_{x=0}(1-\exp(-Fx)-(Fx)\exp(-Fx)]/61$ *where F is force of TB infection and x is age.*

effective contact number) is determined by the ratio of the force of infection and the prevalence of infectious pulmonary TB cases. The ratio between a force of infection of 4 - 8% (4 000 - 8 000/100 000) TB infection rate and a prevalence of smear-positive pulmonary cases notified during 2009 in Cape Town of 370/100 000 indicates an effective contact number of between 11 and 22. Historically, the effective contact number in the UK declined from 22 in 1900, to 10 in 1950 and to 1 in 1990.[13]

For long-term control of an epidemic, the effective contact number must be lower than the number of individuals who can be expected to produce a single new case of infectious pulmonary TB during a lifetime. As the lifetime risk in Cape Town of developing smear-positive pulmonary TB among HIV-negative residents is approximately 10%, the effective contact number must be reduced to <10 to achieve a reduction in the TB epidemic. Current interventions are not achieving this.

Impact of the HIV epidemic

The South African HIV epidemic has expanded markedly over 20 years, and it was recognised early that TB was a major cause of morbidity and mortality.[15,16] A pertinent question is the degree to which the HIV-associated TB epidemic has caused the failure to control TB or the manifestation of the failure to contain TB transmission. Although 44% of the total TB case load in Cape Town in 2009 was HIV-related, only 14.3% of smear-positive pulmonary disease was HIV-related. It therefore appears likely that the HIV epidemic disproportionately increases TB case load rather than TB transmission.

There is no strong evidence that HIV-associated immune suppression affects the acquisition of TB infection (α Fig. 3). Instead, HIV infection appears to be associated with a marked increase in risk of progression from latent infection to TB disease, following either a primary (β_2 Fig. 3)[17-19] or recurrent exposure (β_1 Fig. 3).[20,21] Case-finding proportions (γ Fig. 3) were reduced in HIV-infected individuals (44%) compared with HIV-uninfected (57%) in a Cape Town township.[7] However, the population case-finding proportion improved following introduction of an ART programme (64%).[8] TB case fatality (μ_1 and μ_2 Fig. 3) is increased particularly in those with low CD4 cell counts.[22] ART partially reverses immune suppression[23] and can reduce risk of TB progression after recent TB exposure (β_1 Fig. 3) and, together with isoniazid preventive therapy, can decrease the progression from latent to active disease (β_2 Fig. 3).[24] Active case-finding within ART programmes markedly improves TB case finding.[12,25-28]

Why is TB control failing?

Broad existing strategies for TB control are case-finding and treatment of active disease, treatment of latent TB infection, and vaccination with bacille Calmette-Guérin (BCG).[25] Universal BCG vaccination of all infants protects against progression to miliary TB and TB meningitis but does not affect TB transmission.[29] Treatment of latently infected individuals with isoniazid prophylaxis has not been widely implemented in either HIV-infected or uninfected populations in SA.[2]

The SA TB control strategy predominantly focuses on the quality of case management of patients passively presenting to TB clinics. Passive case-finding is detection of active TB disease among symptomatic patients presenting to medical services, and is promoted in developing countries as part of the WHO-recommended DOTS strategy.[30,31] Consequently, the primary targets and reporting statistics of the SA TB control programme has been the proportion of TB cases which are effectively treated under DOTS with anti-TB chemotherapy.[1,2] SA national DOTS coverage increased from 77% to 100% and the treatment success rate from 61% to 74% between 2001 and 2006.[1,2] However, TB notifications doubled in the same period.[1,2]

The TB burden was decreasing in industrialised countries before effective chemotherapy was introduced, with reductions in the force of infection from approximately 10% to 1% in the early 20th century.[13] Introducing effective chemotherapy in the 1950s consolidated these trends in improved TB control. While effective TB case management is necessary in TB control, it was, however, predicted that it would be insufficient for TB control in scenarios such as South Africa with a high force of infection, high proportion of latently infected individuals and a generalised HIV epidemic.[32] The DOTS strategy is insufficient in high HIV-burdened settings.[33] In high transmission settings where effective contact numbers are high, lower case-finding rates and delays in diagnosis and initiation of chemotherapy result in ongoing transmission.

Development of a new TB control strategy

The benefits of improved case-finding depend on the prevailing epidemiology of TB transmission. In a setting with a force of infection <1.0, detecting a case of TB will mainly benefit that individual alone. In contrast, the benefit of early detection of a TB case where there is an effective contact number >10 will additionally prevent up to 10 secondary cases. The benefits of increased and earlier case-finding on TB transmission are therefore significantly amplified in high-transmission settings. Decreasing TB infection rates is fundamental to achieving the long-term aim of TB control of a steady decline of disease in successive generations. Reducing the high force of TB infection, especially in high-density townships, should therefore become a primary target for long-term TB control. Historical TB control measures using community-based interventions such as enhanced and intensified case-finding strategies must be re-explored in view of the additional benefits accruing for decreasing transmission.[25,34,35]

Reducing the time period of infectiousness also directly influences the prevalence of infectious TB. The period of infectiousness results from delays including health-seeking behaviour, diagnostic delays and health systems delays in initiating effective chemotherapy. Intensified

case-finding can increase awareness of typical symptoms of TB disease, thereby improving health-seeking behaviour. Diagnostic delays can be reduced by using newer molecular diagnostic technologies, and improved health systems efficiencies can further decrease time to initiation of effective TB therapy.

High-risk communities should be specifically targeted, and age-specific interventions are necessary to interrupt TB transmission to infants and young children, school-age children and adolescents, and both HIV-negative and positive adult populations. A change in priority focus from case management to TB transmission reduction should be accompanied by incorporating new outcome measures that reflect ongoing TB transmission at national and sentinel sites. A reduction in TB disease rates among young children, and a steady decrease in the number of latently infected children at school entry and subsequently, would reflect a decrease of TB transmission to children. TB control among adults would be reflected by a decrease in the proportion of recent infections, a decrease in the effective contact number, and eventually a decrease in lifetime risk of TB disease. In HIV-infected patients, full implementation of existing ART guidelines will reduce the pre-ART TB disease burden. The TB infection rates of patients on ART probably reflect the current force of TB infection. The effectiveness of 6-month isoniazid prophylaxis therapy (IPT) to reduce TB disease in already latently infected individuals is very likely undermined where the force of TB infection is high and consistent with southern African data indicating little or no lasting benefit after 6 months IPT.[36,37]

Changing the emphasis from individual benefit to population benefit has parallels with the concept of using ART as prevention, which has been modelled as a potential strategy to control the HIV epidemic.[38,39] This may have an additional effect on control of HIV-associated TB.[40] The HIV force of infection is the result of prevalent community levels of HIV load and sexual networking, which can be reduced by widespread HIV testing and initiation of ART. Similarly for the TB epidemic, the drivers of the high force of infection are population prevalence of infectious TB cases and the effective contact number. Reducing the high force of TB infection, especially in high-density townships, should therefore become a primary target for long-term TB control.

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Antiretroviral Therapy and TB Notification Rates in a High HIV Prevalence South African Community

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Background: Antiretroviral therapy (ART) has been proposed as an intervention for reducing tuberculosis (TB) burdens in areas with high HIV prevalence. However, little data is available on the impact of ART on population-level TB.

Methods: Trends in adult TB case fatality and notifications were assessed before and during increasing ART coverage in a well-defined periurban community, from 1997 to 2008. Mean changes in TB rates were measured using linear autoregression models. ART coverage increased from 1% in 2003 to 5%, 13%, and 21% of HIV-infected population in 2004, 2005, and 2008, respectively.

Results: From 1997 to end of 2004 TB notification rates increased by an average of 187 cases/100,000/year ($P < 0.001$), reaching a peak of 2536/100,000 in 2005. From 2005 to 2008, TB notification rates declined by approximately 202 cases/100,000/year ($P < 0.001$). TB rates were initially stable in HIV-uninfected individuals, but declined moderately from 2005. TB rates declined in HIV-infected adults from 6513/100,000 in 2005 to 4741/100,000 in 2008. The predominant decline in TB notifications occurred among HIV-infected patients receiving ART (1156 cases/100,000/year) and was less marked in those not receiving ART (416cases/100,000/year). Similarly, TB case fatality was constant for HIV-uninfected individuals, but declined in HIV-infected individuals from 23% in 2002 to 8% in 2008 ($P = 0.01$).

Conclusions: In this community heavily affected by both HIV and TB epidemics, rapid and high ART coverage was associated with significant reductions in TB notifications and TB-associated case fatality.

Key Words: antiretroviral, community, HIV, notification rates, tuberculosis

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INTRODUCTION

The current World Health Organization (WHO)–recommended TB control strategy is failing to control the tuberculosis (TB) epidemic in high HIV prevalence countries, and TB rates continue to escalate in countries such as South Africa.[1] The STOP TB Partnership has proposed adjunctive strategies to address this problem, including the "Three I's" strategy for reducing the burden of TB in HIV-infected patients: intensified case finding, isoniazid preventative therapy, and infection control.[2] However, this strategy has not been widely implemented to date. Due to the reduction in TB risk in HIV-infected patients on highly active antiretroviral therapy (HAART),[3,4] HAART has also been proposed as an adjunctive strategy for controlling the TB epidemic in low-income and middle-income countries with generalized HIV epidemics.[5] This strategy has been more extensively implemented, and through programs such as the "3 by 5" initiative,[6] the President's Emergency Plan for AIDS Relief (PEPFAR),[7] and the Global Fund,[8] there has been substantial progress in patients' access to HAART.[9]

However, the impact of HAART on TB rates at a population level remains uncertain. Although incidence of active TB disease in HIV-infected patients is reduced by 70%–90% by HAART,[3,10] patients still have a 5–10 times higher risk of TB disease 3 years into HAART treatment compared with HIV-uninfected individuals.[11,12] The combination of prolonged survival and residual increased risk of TB incidence in HIV-infected patients on HAART will result in an increased number of highly susceptible individuals in the population. Therefore, even substantial population coverage with HAART may have a limited impact on TB incidence at a population-level. However, empirical data addressing this issue are sparse, and most evidence comes from mathematical modeling.[13]

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Despite the well-described TB benefits of HAART use for HIV-infected individuals, there are no population-level studies describing the impact of increased access to HAART on community TB rates in areas with generalized HIV epidemics. Therefore, we assessed the impact of increasing antiretroviral provision on TB notification rates in a community with high HIV prevalence.

METHODS

Study Community

The study took place in a well-defined South African periurban township with a population of approximately 15,000 people and an HIV prevalence of 23% in 2005.[14] The community is served by a single primary care clinic that follows the National TB control program guidelines,[15] based on WHO-recommended DOTS program. The clinic manages all TB patients resident in the community, and the protocol for diagnosis and management of TB patients did not change significantly from 1997 to 2008. The main change to the national TB protocol has been the addition, since 2004, of active TB screening for patients initiating HAART. This screening was based on sputum investigation and did not include testing for latent TB infection. Isoniazid preventive therapy has not been implemented in this community. Despite an apparently well-functioning TB program,[16] we have previously reported escalating TB notification rates in this community before rapid, high coverage HAART availability.[17] HAART provision began in 2003 with patients in the community accessing antiretroviral treatment at the local clinic or local hospital, but the HAART program was only scaled-up in 2005.

TB and HAART Data

TB notification data were obtained from the local TB clinic from 1997 to 2008. HIV status, antiretroviral treatment status, and CD4 count data were obtained from the TB register, clinical folders, and clinic and hospital HAART databases. Adults were defined as patients ≥15 years of age.

Population Model

Annual and age-specific TB rate calculations were based on population denominators obtained from the 1996 South African national census, and community household censuses performed in 2002, 2004, 2006, and 2008. Linear population growth was assumed between each census. Community HIV prevalence from 1996 to 2004 was estimated using the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model for the African population.[18,19] In 2005 and 2008, the Desmond Tutu HIV Centre performed two community-based random cross-sectional HIV prevalence surveys among adults ≥15 years of age in the study community.[14,20] When the ASSA 2003 model predictions were compared with the 95% confidence intervals (CIs) from the community surveys, the 2005 survey data[14] matched the model's predictions for the HIV prevalence in the community. However, the 2008 survey showed a shift toward higher HIV prevalence among 30 to 45-year olds and particularly among women. This is in keeping with the impact of antiretroviral programs on community HIV epidemics, and the ASSA model estimates for the study community HIV prevalence from 2005 to 2008 were adjusted based on the survey results. The number of HIV-infected individuals derived from this model was used as the denominator for calculation of TB rates among HIV-infected population. Numbers of patients on HAART in each year, derived from the antiretroviral program registers, were used as denominators for TB notification rates on HAART. HAART coverage was calculated as the proportion of the adult HIV-infected population in the community receiving HAART in each year.

HIV and TB Rates Calculations

All TB rates are reported as cases/100,000. HIV testing was routinely offered to TB patients from 2002, and therefore, HIV-associated rates were only available from that year. To account for missing HIV test results, extreme case scenarios for HIV-associated and non–HIV-associated TB rates were calculated assuming 100% of patients with unknown HIV status were HIV infected and HIV uninfected, respectively. Analyses in HIV-infected and HIV-uninfected strata were performed on patients with known HIV status.

Direct standardization method was used to calculate the age-standardized annual TB rates for the population using the 1997 population age distribution as the reference population. Similarly, using HIV-infected population as the reference population, direct standardization was used to calculate age-standardized rate ratio (RR) of TB in HIV-infected adults off HAART versus adult patients on HAART. Mean changes in TB rates and significance of trends before (pre-2005) and after (post 2004) the implementation of a high coverage HAART program were assessed using linear regression models. An autoregressive model with a 1-year lag was used to account for the autocorrelation of the data, and the impact of HAART was examined through an interaction term. We a priori chose 2005 as the first year of HAART availability, as this was the first year that an appreciable number of patients were receiving HAART. The median baseline CD4 counts of patients initiating HAART in each year were calculated from the most recent CD4 count in the 3 month period before HAART initiation.

TB completion rates were calculated as proportion of TB patients who completed TB treatment (TB treatment completion or cure), excluding those who were transferred out of the community during the treatment course. Retreatment TB was defined as a new TB diagnosis in a patient who had previously completed TB treatment. All cause case fatality rates in TB patients were calculated as proportion of TB patients who died on TB treatment each year, excluding those who were transferred out of the community during the treatment course. Trend analysis for TB treatment completion rates and case fatality rates were assessed using χ^2 test for trend.[21]

Data were analyzed using Stata Version 10.0 (Stata Corporation, College Station, TX). All statistical tests were 2-sided at alpha = 0.05. This study analysis was performed on register data, and ethics approval for the study was obtained from the University of Cape Town Human Research Ethics Committee.

RESULTS

Over the 12-year study period, 1974 TB cases were notified in the study community. Of these, 1741 were adult cases, the median age of which was 32 years (interquartile range: 26–40 years), and 45% were female. Overall, HIV testing was performed in 77% of adult cases, of which 69% were HIV infected. From 2002, 87% of adult TB cases were tested for HIV, of which, 70% were HIV infected. In 2003, 1% of the HIV-infected population was receiving HAART. By end of 2005, this proportion had increased to 13%, and by the end of the study period, 21% of the estimated HIV-infected population were receiving HAART (Fig. 1).

Adult TB Notification

Table 1 shows total adult TB notification, TB case fatality, and TB treatment completion data over the study period. From 1997 to end 2004, adult TB notification rates (per 100,000) increased by an average of 187 cases a year (95% CI: 143 to 232; $P < 0.001$). From 2005, the rate of adult cases decreased by an average of 202 cases per year (95% CI: −233 to −172; $P < 0.001$) (Fig. 1). The annual age standardized rates confirmed these trends, with an increase in annual rate from 1998 to 2005, followed by a decreasing trend to 2008 (Table 1). TB treatment completion rates averaged 80% over the study period, with no significant change over the study period ($P = 0.88$).

HIV-Infected and HIV-Uninfected TB Notifications

Table 2 shows TB notification data for HIV-uninfected patients and HIV-infected patients not on HAART and those patients on HAART at time of TB diagnosis from 2002 to 2008. Adult TB rates in HIV-infected and HIV-uninfected TB patients are shown in Figure 2A, including extreme case scenarios for HIV-associated and non–HIV-associated TB rates assuming 100% of patients with unknown HIV status were HIV infected and HIV uninfected, respectively. TB rates (per 100,000) in known HIV-uninfected patients did not change substantially from 1997 to 2004 (with an average annual increase of 49 cases; $P = 0.65$), but showed a modest annual decline of 143 cases per year from 2005 (95% CI: −195 to −91; $P < 0.001$). However, from 2002 to the end of 2004, overall TB rates in known HIV-infected adults increased by an average of 432 cases per year (95% CI: 109 to 755; $P = 0.009$), after which rates decreased significantly by 578 cases per year (95% CI: −697 to −459; $P < 0.001$). TB treatment completion rates did not change significantly for HIV-infected or HIV-uninfected patients from 2002 to 2008 ($P = 0.21$ and $P = 0.43$, respectively).

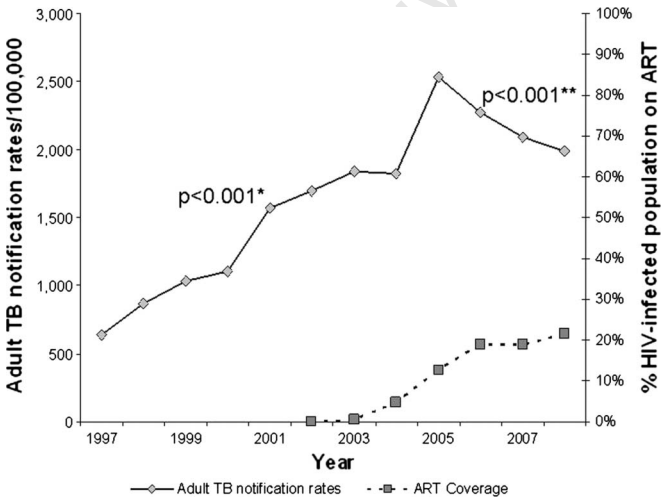

FIGURE 1. Adult TB notifications rates and antiretroviral coverage in study community, from 1997 to 2008. *The P value for autoregression model from 1997 to 2004. **The P value for autoregression model from 2005 to 2008.

TB Notification and HAART

Figure 2B shows TB rates (per 100,000) in HIV-infected patients stratified by HAART. TB rates in HIV-infected patients not on HAART increased by an average of 362 cases per year from 2002 to end 2004 (95% CI: −264 to 988; $P = 0.26$). From 2005, there was a significant average annual decrease of 416 TB cases in HIV-infected patients not on HAART (95% CI: −526 to −305; $P < 0.001$). TB rates in HIV-infected patients on HAART decreased significantly by an average of 1156 cases per year (95% CI: −1191 to −1120; $P < 0.001$). After standardizing for age differences across annual populations, HIV-infected patients not on HAART had a lower rate of TB compared with HIV-infected patients on HAART early in the HAART program (RR = 0.41 in 2004). However, as the period of the HAART program increased, the RR of TB in patients off HAART increased to nearly twice that of the patients receiving HAART (RR = 1.98 in 2008).

Baseline CD4 Count

The number of HIV-infected patients initiating HAART in each year is shown in Table 2. The median baseline CD4 counts in patients commencing HAART increased from 15 cells per microliter in 2003 to 86 cells per microliter in 2004, 129 cells per microliter in 2005, peaking at 153 cells per microliter in 2006, and then stabilizing at 122 cells per microliter in 2007, and 141 cells per microliter in 2008.

Retreatment TB

Overall retreatment TB rates (per 100,000) increased significantly from 1997 to 2004, at an annual average increase of 52 cases (95% CI: 35 to 71; $P < 0.001$), after which retreatment rates stabilized ($P = 0.59$). Retreatment TB rates in HIV-uninfected patients remained stable over the study period ($P = 0.53$ from 2002 to end 2004 and $P = 0.56$ from 2005 to end 2008). In HIV-infected patients not receiving HAART, retreatment TB rates increased by an average of 157 cases per year (95% CI: 95 to 219; $P < 0.001$) from 2002 to 2004, and then decreased by an annual average of 67 cases (95% CI: −129 to −5; $P = 0.03$). Annual retreatment TB rates in HIV-infected patients on HAART from 2004 to 2008 decreased significantly by an average of 824 cases/100,000 per year (95% CI: −1492 to −156; $P = 0.02$) (Fig. 3).

TB Case Fatality

Overall case fatality during TB treatment decreased over the study period (Fig. 4). Although TB case fatality rates

TABLE 1. Total Adult TB Notification and Age-Standardized TB Rates, and TB Case Fatality and Treatment Completion Rates in the Study Community, from 1997 to 2008

Year	Number of Adult TB Cases			Adult Population	Total TB Rates/100,000	Age-Standardised TB Rate/100,000	Case Fatality (%)	Treatment Completion (%)
	Total TB	TB Case Fatality	TB Treatment Completion					
1997	30	2	17	4695	639	Reference population	7	61
1998	46	1	32	5305	867	882	3	80
1999	61	1	53	5916	1031	1060	2	98
2000	72	7	47	6527	1103	1117	11	77
2001	112	9	75	7138	1569	1582	10	80
2002	131	9	94	7722	1696	1762	8	80
2003	160	17	122	8714	1836	1876	11	79
2004	177	17	125	9706	1824	1924	11	81
2005	252	20	176	9935	2536	2784	9	79
2006	231	15	170	10,165	2273	2386	7	78
2007	231	13	181	11,062	2088	2171	6	85
2008	238	10	163	11,958	1990	2183	5	81

remained relatively constant in HIV-uninfected individuals ($P = 0.81$), TB case fatality dropped significantly in HIV-infected patients from 13% in 2002 to 4% in 2008 ($P = 0.001$).

DISCUSSION

This community-based study from sub-Saharan Africa demonstrated an association between the implementation of an HAART program and TB notification rates. In this community, TB notification and case fatality rates decreased with the rapid and high coverage implementation of an HAART program.

TB is a major cause of mortality in HIV-infected patients in sub-Saharan Africa.[1,22] A key finding in this study is the significant reduction in TB case fatality rates in HIV-infected patients. The use of HAART has been associated with a reduction in TB-associated mortality among individuals and in treatment cohorts.[23,24] In our study, the implementation of a community HAART program was also associated with a decrease in TB case fatality in HIV-infected patients. In 2002, the case fatality rate in HIV-infected patients was 4 times greater than that of HIV-uninfected patients but had decreased to the same rate as HIV-uninfected patients by 2008. This suggests that HAART programs may greatly assist in achieving the Stop TB Partnership goal to halve TB mortality rates by 2015.

The decrease in TB notification rates in the study community occurred against a background of increasing national TB notification rates.[1] TB rates in HIV-uninfected patients were stable from 1997 to 2004, with HIV-associated TB driving the escalating epidemic in the study population. Although HIV-uninfected rates showed a decline from 2005, the reduction in community TB notification rates in this study was predominantly due to a decrease in TB rates in HIV-infected patients and more specifically in patients receiving HAART.

Although the HIV prevalence in this community was relatively stable from 2002 to 2008, the TB notification rates in HIV-infected patients continued to escalate until 2005. After the rapid scale-up of HAART availability from 2005, the TB rates in HIV-infected patients not on HAART stabilized

TABLE 2. TB Notification Rates Among Adult HIV-Uninfected and HIV-Infected Patients in the Study Community, from 1997 to 2008

Year	Number of Adult TB Cases			Adult Population HIV Prevalence	Adult HIV-Infected Population	Adult HIV-Infected Population on HAART	Adult TB Rates/100,000		
	HIV Uninfected	HIV-Infected off HAART	HIV-Infected on HAART				HIV Uninfected	HIV-Infected off HAART	HIV-Infected on HAART
2002	39	70	0	22.5	1737	0	652	4030	0
2003	50	85	0	22.9	1993	11	744	4289	0
2004	52	100	8	23.0	2233	113	696	4697	7692
2005	81	130	20	23.2	2303	308	1,061	6458	6897
2006	52	121	27	25.5	2591	516	687	5762	5499
2007	50	126	25	26.0	2877	573	611	5408	4570
2008	52	129	21	26.5	3164	713	591	5193	3088

Patients of unknown HIV status excluded.

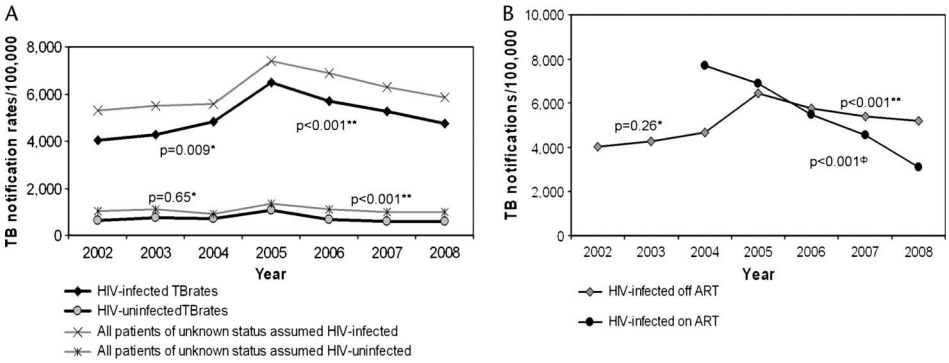

FIGURE 2. TB rates in HIV-uninfected and adult HIV-infected patients (A) and HIV-infected patients receiving HAART and not receiving HAART (B) in the study community, from 2002 to 2008. *The *P* value for autoregression model from 2002 to 2004. **The *P* value for autoregression model from 2005 to 2008. ϕThe P value for autoregression model from 2004 to 2008.

and then decline moderately. However, there was a dramatic, almost 3-fold decline in TB rates in those HIV-infected patients on HAART. Of note is the high initial retreatment TB rate in patients receiving HAART compared with those patients not on HAART, followed by a substantial reduction in retreatment TB in HIV-infected patients on HAART.

The levelling-off and subsequent decline of TB notification rates in HIV-infected patients not on HAART may be due to the removal of those with the highest TB risk (lowest CD4 counts) from this group into the group on HAART.[4] This stabilizing of notification rates suggests that the rate at which people are removed from the susceptible pool approximates the rate at which HIV-infected patients not on HAART are progressing into a high-risk state of immune compromise. This finding suggested that the rate at which an HAART program is implemented in a community might be an important variable determining the impact of this intervention on overall HIV-associated TB rates.

Although TB does occur at all CD4 strata, the highest risk of TB in HIV-infected patients occurs at low CD4 counts (<200 cells/μL).[25,26] The South African National Antiretroviral program recommended initiating patients on HAART at CD4 count <200 cells per microliter or WHO clinical stage IV.[27] Patients initiating HAART have a high risk of TB in the first months of treatment compared with later in treatment due to the risks associated with low baseline CD4 counts and with possible unmasking of subclinical TB.[28–30] The overall increase in median baseline CD4 count in our study reflects that patients with more advanced disease were initiated onto treatment in the early stages of the HAART program. With increasing duration of the HAART program, the pool of severely immune-compromised patients off treatment decreased, and patients were started on HAART at higher baseline CD4 counts. However, despite the overall increase in median baseline CD4 count, by 2008, the median CD4 count at initiation of HAART was still well below 200 cells per microliter and TB risk before HAART initiation remained high, as evidenced by the nearly 2-fold higher standardized RR in HIV-infected patients off HAART compared with those patients on HAART in 2008.

HIV-infected patients off HAART accounted for 64% of TB disease in 2008, and thus contribute a large portion of the TB burden. By 2008, the HAART coverage in this community was high, at approximately 90% of the estimated community need as defined by the national HAART treatment guidelines.[27,31] If similar coverage rates and impacts were achieved nationally, a >20% reduction in TB rates might be attained. But this impact could be more substantial if treatment was initiated earlier in the HIV disease process, thus also reducing

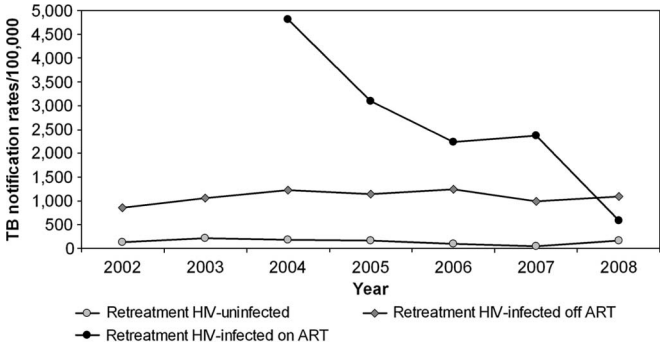

FIGURE 3. Adult retreatment TB rates overall and by HIV and HAART status, in the study community from 2002 to 2008.

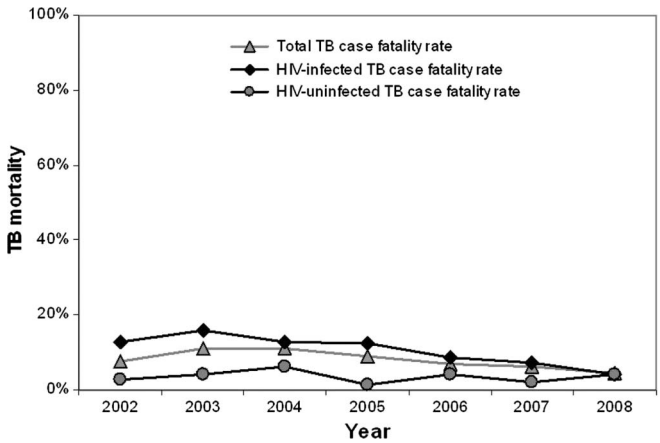

FIGURE 4. Adult TB case fatality rate by HIV status, in the study community from 2002 to 2008.

TB burden before HAART initiation and during the early months on HAART.

Prevalence of TB is a function of TB incidence and period of infectivity in the community. Period of infectivity can be decreased by earlier diagnosis and treatment of cases. The key change to case-finding activities has been the introduction of active case finding for TB in patients initiating HAART. This practice may have resulted in a decrease in TB prevalence, thus indirectly contributing to the decrease in notifications over time. In addition, in 2005, a community-based cross-sectional survey was performed in 10% of the study community, investigating participants for active TB disease. A sensitivity analysis, excluding those participants diagnosed in that survey from the notification data, showed no change in the study results, including the peak noted in 2005 (data not shown). Therefore, this survey does not seem to have had a direct effect on the notification rates in this community. The increased TB screening of HAART-eligible patients associated with the scale-up of the HAART program in 2005 may explain the 2005 peak in notification rates. This increased screening together with the survey may have increased community awareness of TB, potentially contributing to the moderate increase in TB notifications also noted in HIV-uninfected patients.

Although there was a strong temporal association between the decline in TB notification rates and the implementation of an HAART program and there is substantive biological plausibility to support this association, it is worth considering alternative explanations for the decrease noted in TB notification rates. Improved TB treatment completion rates may, for example, result in decreased TB transmission, which could reduce TB incidence. However, TB completion rates remained stable over the study period, and this was consistent for both HIV-infected and HIV-uninfected patients. Period of infectivity, and thus prevalence could be decreased by increased TB-associated mortality; however, TB case fatality has decreased.

There were no changes in the infection control policies within the clinic over this period, and therefore, decreased nosocomial TB transmission is unlikely to explain the study findings. Although changing social conditions may also impact TB transmission in communities, this community has remained one of extremely poor socioeconomic status since its establishment in 1994.

HIV testing uptake was not complete among TB patients in this community. However, to explore the potential biasing influence of those patients with unknown status, we performed an extreme case scenario analysis. The result of this analysis showed that our findings were robust and did not alter the findings of the study. The population denominators were derived from community census data with the assumption of linear growth between censuses, and HIV-infected denominators were obtained from a mathematical model fitted to local HIV data. Sensitivity analyses were run for models assuming different trends of population growth and different assumptions for HIV prevalence. These analyses also did not show substantive changes in study inferences. This community is typical of many recently urbanized populations in South Africa, but further investigations are needed to confirm the generalizability of our findings in other high prevalence settings.

This study was performed in a well-demarcated community, with population data derived from frequent community censuses. All residents receive their TB treatment at a single clinic, and therefore, TB notification data is likely to be a complete representation of TB notifications in the community. Similarly residents obtained HAART from the clinic or the local referral hospital and access to both these databases ensured an accurate description of the HAART program in this community. These analyses are dependent on the fidelity of the relevant data records, and therefore, the databases underwent a 10% quality control assessment.

In conclusion, against a background of increasing TB notifications nationally, we have shown that a rapidly implemented, high coverage HAART program can reduce the TB notification rates and TB case fatality within a community heavily affected by both HIV and TB epidemics. This reduction in TB was due predominantly to the decrease in TB rates in HIV-infected patients receiving HAART and may be the result of both active TB screening and improved immune function in these patients.

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Transmission Elasticity in Communities Hyperendemic for Tuberculosis

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Backround. Despite consistently meeting international performance targets for tuberculosis case detection and treatment success, areas where tuberculosis is hyperendemic fail to achieve the predicted epidemiological impact. In this article, we explore the anomalous relationship between defined performance targets and actual reduction in tuberculosis transmission.

Methods. In areas where tuberculosis is endemic, poorly ventilated social gathering places such as shebeens (informal alcohol drinking places), minibus taxis, and clinic waiting rooms are all potential transmission hot spots. We modeled the transmission reduction achieved by removal of infectious persons in settings with different tuberculosis prevalence rates to demonstrate the concept of transmission elasticity. We then applied this concept to real-life data from a hyperendemic community in Cape Town, South Africa.

Results. In a hyperendemic area, reducing the number of infectious people by a given percentage results in a smaller percentage decrease in the annual risk of infection (ARI) compared with a nonendemic area; for example, removing 10% of infectious persons could result in as little as a 5% reduction in the ARI. With use of real-life data and removal of 60% of infectious individuals with tuberculosis, as would be achieved by meeting current performance targets of 70% case detection and 85% cure, the estimated ARI reduction is 50%.

Conclusions. The relationship between the number of infectious people removed and the decrease in ARI is nonlinear. The concept of transmission elasticity has important implications for the formulation of universal performance targets, since hyperendemic areas would require more stringent targets to achieve comparable transmission reduction.

Tuberculosis is caused by *Mycobacterium tuberculosis*, which spreads by aerosol transmission. Individuals with active lung disease expel infectious droplets into the ambient air, where they remain suspended for a considerable period in the absence of adequate ventilation. Progression to disease is uncommon among immunocompetent people following inhalation of an infectious droplet. However, high levels of ongoing transmission and sufficient numbers of vulnerable people sustain the epidemic in areas where tuberculosis is endemic [1–3]. Molecular epidemiology studies have established that in areas where tuberculosis is endemic, disease results from recent infection (primary or reinfection) in the majority of cases [1–3]. This underscores the importance of reducing ongoing transmission in order to establish epidemic control.

The World Health Organization adopted the Directly Observed Therapy Short course (DOTS) strategy together with specified performance targets in order to control the tuberculosis epidemic. Despite curing millions of patients with tuberculosis and saving many lives, the epidemiologic impact of this strategy has been mixed [4, 5]. The global tuberculosis incidence (number of new cases identified each year) shows excessive variability, with the highest rates being recorded in sub-Saharan Africa. Tuberculosis incidence rates in excess of 1,500 cases per 100,000 people have been reported in some hyperendemic communities. Mathematical

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models based on data from Western Europe predicted that achieving 70% case detection and 85% cure rates would lead to a decrease of tuberculosis incidence of up to 11% per year [5–7]. However, tuberculosis incidence failed to decrease as predicted in many hyperendemic areas where the DOTS strategy has been fully implemented and performance targets met [5, 8]. This failure could be attributed to inaccurate calculation and/or reporting of estimates or to deficiencies in the mathematical models used to define DOTS-related performance targets. Applying mathematical models in regions other than those from which they were derived is questionable, since these models fail to consider transmission dynamics that may be unique to hyperendemic areas. Typical models assume homogeneity; that is, they assume that infectious people are uniformly distributed over the whole population, but in reality tuberculosis cases are heavily clustered within particular sections of the community. In this article, we explore the potential nonlinearity between the number of infectious tuberculosis cases removed (successfully treated) and the decrease in ongoing tuberculosis transmission, as a novel reason for unreliable predictions by standard models when applied to hyperendemic areas.

Settings that are most conducive to transmission are crowded and poorly ventilated households, workplaces, prisons, and homeless shelters as well as shebeens (informal alcohol drinking places), minibus taxis, clinic waiting rooms, community halls, and churches [9–11]. For convenience, we use the term *cohort* to mean a group of people who spend time together in such a setting on a regular and frequent basis. For our purposes, the cohort need not comprise the same people each time. Susceptible people may be members of several cohorts; for example, they may undertake a minibus taxi journey to work in the morning (transport cohort), spend several hours at work (work cohort), and then return home in the afternoon with a second transport cohort. In hyperendemic areas around Cape Town, South Africa, many unemployed people spend a great deal of time socializing in shebeens [9, 10]. The essential attributes of a cohort entail spending prolonged time on a regular basis with a group of people, approximately fixed in number, in an enclosed space with inadequate ventilation.

In any given cohort, infectious tuberculosis cases may be present, in which case the cohort would become epidemiologically active. In hyperendemic areas, it is expected that, not infrequently, 2 or more concurrent infectious persons with tuberculosis will form part of a single cohort (doublet cohort), whereas this would be extremely unlikely in low-prevalence areas, where only 1 infectious person would form part of a cohort (singlet cohort). Treating 10% of infectious people in a low-prevalence setting will therefore cause, on average, nearly 10% of the epidemiologically active cohorts to be deactivated so that one can expect a 10% decrease in transmission. This will not be the case in hyperendemic areas, where doublet or multiple exposure cohorts will remain active since they are likely to lose only 1 of the $\geq$2 infectious members. The expected decrease in transmission will therefore be $<$10%.

We utilized established transmission theory and real-life data from a well-characterized community to quantify the above considerations and show that in similar settings the relationship between the number of infectious tuberculosis cases successfully treated and the decrease in the annual risk of infection (ARI) is indeed unequal. Thus, reducing the number of infectious people by a given percentage results in a smaller percentage decrease in the ARI than might otherwise be expected. This has important implications for setting optimal performance targets for tuberculosis control programs.

METHODS

To quantify the transmission reduction achieved by successfully treating infectious tuberculosis cases, we have to estimate the following: (1) the probability distribution of infectious people among different cohorts, (2) the expected probability of transmission events in cohorts containing variable numbers of infectious people, and (3) for a given cohort, the risk of a susceptible individual becoming infected or reinfected over the course of 1 year, before and after a given percentage reduction in the number of infectious people.

Probability Distribution of Infectious People Among Cohorts

The cohorts most conducive to transmission involve prolonged socialization in crowded places with poor ventilation. Any of these cohorts may be shared by 2 or more infectious people. In general, we must find the distribution of infectious people among these cohorts and estimate the proportion of cohorts (of each type) that actually contain $\geq$2 infectious persons.

Suppose that in a community of M people there are N who have infectious tuberculosis. The probability that any given person in the population is infectious is given by $\rho = N/M$. Assuming independence in the distribution of the infectious people, the probability, P_k, that there are k infectious people in a cohort where X people are present can be approximated by a Poisson distribution with parameter $\lambda = \rho X$:

$$P_k = \frac{\lambda^k}{k!}\exp(-\lambda) \quad (1)$$

As an example, we use data from a well-characterized hyperendemic community [12]. Figure 1 reflects the percentage of cohorts likely to contain either 1 or 2 infectious tuberculosis cases in this community, providing for different cohort sizes. For example, with a tuberculosis prevalence of 1,600 cases per 100,000 people and cohort size 20, ~4% of the cohorts will contain 2 infectious people (doublet cohorts) and 24% will

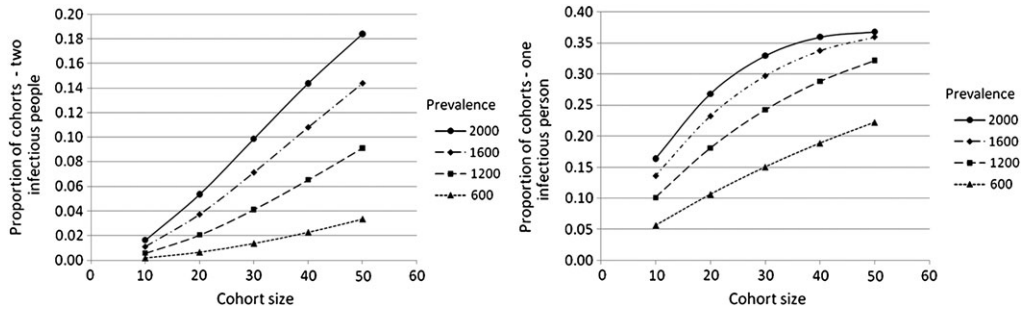

Figure 1. Distribution of infectious people among community cohorts at various tuberculosis prevalence rates (cases per 100,000 people per year).

contain only 1 (singlet:doublet ratio of 6:1). With larger cohort sizes the relative number of doublet cohorts increases. The number of cohorts containing ≥3 infectious people seems negligible (with $M = 15{,}000$ the expected number of triplet cohorts is .8), so we ignored this possibility in our model; triplet cohorts may occur more frequently with exceptional case densities.

Transmission Risk Within a Cohort Containing a Given Number of Infectious People

The circumstances conducive to tuberculosis transmission are those of close confinement in poorly ventilated areas for prolonged periods. Being a member of an active cohort implies tuberculosis exposure and the possibility of acquiring infection. The number of transmission events within each cohort can be estimated using the Wells-Riley equation [13–15] or the Gammaitoni and Nucci model [16]. Under the conditions we are considering here there is little difference in the predictions made by these 2 models [17, 18], and we elect to use the Wells-Riley model:

$$\frac{C}{S} = \left(1 - e^{-kpTq/Q}\right) \tag{2}$$

In this equation, C is the number of transmission events, S is the number of susceptible people, k is the number of infectious people in each cohort, T is the time of exposure, q is the infectivity of the infectious people, p is the susceptible respiration rate, and Q is the germ-free ventilation.

As an example, using typical data from our well-defined study community [12], $S = 18$ (for 2 infectious people in a cohort of size 20), $q = 1$, $T = t \times D$, where $t = 1$ h/d and $D = 60$ d is the period of infectiousness, and $P = 14{,}400$ L/d. Figure 2 reflects the probability of transmission within cohorts, depending on the number of infectious cases and various ventilation rates (Q). For a Q value of 2 m^3/h, the probability of transmission when 1 infectious person is present in a cohort is .46, whereas for 2 infectious people in a cohort, the probability is .71.

Transmission Reduction Resulting From Removal of Infectious Cases

We assume passive case finding, in which cases emerge from the community in a random way with equal chances of treatment success. We calculate the proportional reduction in transmission events after the removal, in a random manner, of a given proportion of infectious cases as follows.

The expected annual risk of infection within a cohort is given by the expected proportion of the year during which 1 infectious person is present in the cohort multiplied by the probability of transmission when 1 infectious person is present plus the similar factor for 2 infectious people. This annual risk is therefore specified by the following formula:

$$R = \sum_{k=1}^{2} \frac{\lambda^k}{k!} \exp(-\lambda).\left(1 - \exp\left(-\frac{kpTq}{Q}\right)\right) \tag{3}$$

The probability that 3 or more infectious people are present is negligible and is therefore ignored.

For convenience, we write equation (3) as follows:

$$R = \sum_{k=1}^{2} \frac{\lambda^k}{k!} \exp(-\lambda).(1 - \exp(-k\phi)) \tag{4}$$

We require the proportional effect on R when the number of infectious people (ie, the prevalence) is reduced by a given proportion. This corresponds exactly to the concept of price elasticity of demand in economics, and we therefore find it appropriate to use the prevalence elasticity of annual risk of infection or transmission elasticity given by the following:

$$\eta = \frac{dR}{d\rho}.\frac{\rho}{R} \tag{5}$$

Applying equation (5) to equation (4) and using $\lambda = \rho X$ (equation [1]) we find the following:

$$\eta = \frac{(1-\lambda) + \left(\lambda - \frac{\lambda^2}{2}\right)\theta}{1 + \frac{\lambda}{2}\theta} \tag{6}$$

where $\theta = 1 + e^{-\phi}$ with $\phi = pTq/Q$ as above.

Note that $\eta = dR/d\rho \cdot \rho/R$ (equation [5]) is the limiting value of $\Delta R/\Delta\rho \cdot \rho/R$, where ΔR and $\Delta\rho$ are (small) increments

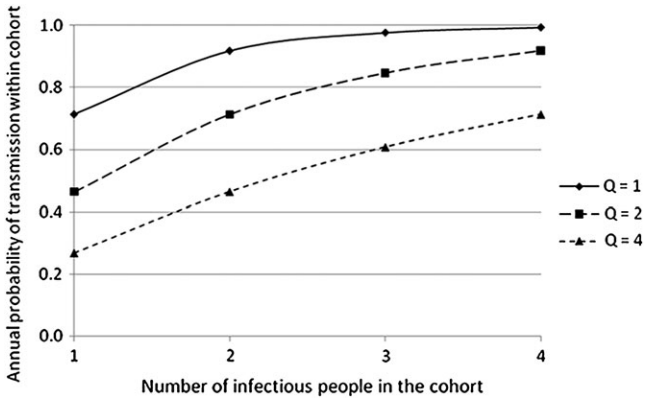

Figure 2. Estimated annual probability of transmission within a given cohort, of size 20, depending on the number of infectious people in the cohort and various ventilation rates (Q). daily exposure time (t), 1 h/d; period of infectiousness (D), 60 d [12]; susceptible respiration rate (p), 14,400 L/d.

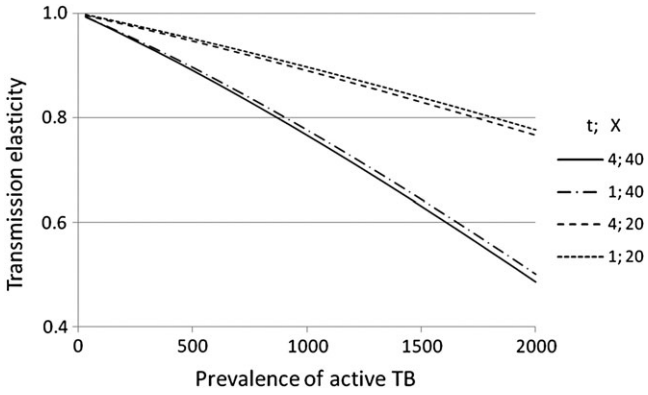

Figure 3. Proportional reduction in annual risk of infection compared with tuberculosis (TB) prevalence within cohorts of different size ($X = 20$ or 40 people) and different daily exposure times ($t = 1$ or 4 h). period of infectiousness (D), 60 d [12]; susceptible respiration rate (p), 14,400 L/d; ventilation rate (Q), 4 m^3/h.

in R and ρ, respectively. We observe that $\Delta R/\Delta\rho \cdot \rho/R$ can be written as

$$\frac{100^{\frac{(-\Delta R)}{R}}}{100^{\frac{(-\Delta\rho)}{\rho}}},$$

where the minus signs indicate decreases rather than increases. Thus,

$$\left(100^{\frac{(-\Delta R)}{R}}\right) \sim \eta\left(100^{\frac{(-\Delta\rho)}{\rho}}\right),$$

that is, the percentage reduction in the ARI is approximately η times the percentage reduction in the prevalence.

Transmission elasticity reflects the potential effectiveness of attempts to reduce transmission by case detection and treatment. In the ideal situation where $\eta = 1$, the percentage reduction in ARI is exactly equal to the percentage reduction in prevalence. This is implicitly assumed in most mathematical models where the law of mass action is applied to estimate the rate of transmission (rate of transmission = force of infection $\times$ number of infectious people $\times$ number of susceptible people). We are concerned here with the possibility of settings where $\eta < 1$. At a low prevalence the elasticity is close to 1, but in hyperendemic communities this may drop significantly below 1. With a value of .6, the percentage reduction in transmission (ARI) will be only 60% of the percentage reduction in prevalence, since ARI reduction is η times the percentage reduction in prevalence.

RESULTS

Figure 3 shows variable rates of transmission reduction (transmission elasticity) at different tuberculosis prevalences within cohorts of size 20 and 40. Figure 3 and formula (6) show that $\eta \to 1$ as $\lambda \to 0$; that is, at low prevalences there is parity between transmission reduction and the percentage reduction in prevalence. Transmission elasticity is demonstrated at high prevalences, where parity with the percentage reduction in prevalence is no longer maintained. At a tuberculosis prevalence of 2,000 cases per 100,000 people the ratio (reflected on the y-axis) drops to <.5, which corresponds to a proportional reduction in ARI that is less than half of the proportional reduction in tuberculosis prevalence.

In order to estimate the effect on the ARI within a community where tuberculosis cases are successfully treated, it is necessary to supply parameter values for formula (6). These parameter values are specific to the community under consideration and also vary from one cohort type to another. We consider 2 population components in order to accommodate variable exposure risk. The high-risk component includes people who spend a minimum of 1 h/d in the company of ≥20 people in circumstances conducive to *M. tuberculosis* transmission. Examples of such conditions include shebeens, taxis, clinics, and community halls; it is estimated that 39% of the study population would fall into this high-risk component (Robin Wood, personal communication). Restriction fragment length polymorphism data suggest that the majority of transmission occurs within this high-risk component where the tuberculosis prevalence is estimated to be >3,200 cases per 100,0000 people [12]. Household transmission is relatively less common than in low-prevalence areas [12, 19], and household cohorts were excluded from this analysis. For simplicity we assumed zero infection risk in the low-risk component, which should result in more conservative estimates for the community as a whole.

We assigned the following typical values: $q = 1$, $T = t \times D$, where $t = 1$ is the minimum time expressed in hours per day and $D = 60$ d is the period of infectiousness, $P = 14{,}400$ L/d, and $Q = 4$ m^3/h (96,000 L/d) [12]. We then calculated the risk of infection for the high-risk component using formula (4). The risk for the remainder of the population is, as explained above, assumed to be 0. The risk of infection for these 2 components

combined is then the weighted sum of these 2 risks with the weightings given by the total proportions that each of these groups form within the community as a whole, namely, 39% and 61%. We now estimate the transmission elasticity in the population as a whole by considering this weighted sum of the risks.

To illustrate the result, we consider the effect on transmission, in the community as a whole, of a 10% reduction in prevalence. With the conservative assumptions made, a 10% case reduction at a prevalence of 1,600 tuberculosis cases per 100,000 people, estimated transmission would be reduced by 8.3% (Figure 4). Given current performance targets (70% case detection and 85% treatment completion), it is of interest to estimate what the transmission impact would be if these targets were achieved and 60% ($.7 \times .85 = .595$) of tuberculosis cases cured. Figure 5 demonstrates the variable ARI reductions achieved given different case densities (initial prevalence rates).

Several points should be noted:

1. The proportional reductions calculated are for the infection rate, not the disease rate. However, the risk to progress from infection to disease should remain constant in the short term, and thus the proportional reduction in disease rate will be similar. However, if the population profile changes considerably over time (reducing overall vulnerability) then correspondence may no longer apply. These factors should be accounted for in mathematical models of the longer-term epidemiological impact where, in the typical scenario, the state variables are updated using successive time-step iterations. These changing values imply a possible change in risk to progress from infection to disease. The changes may be small at each time step, but the cumulative effect could be substantial.
2. Our calculations reflect the direct impact on transmission; they do not consider secondary or more dynamic longer-term effects. When modeling longer-term impact, various delays have to be factored in; delays that facilitate ongoing transmission will only accentuate the elasticity effect.
3. In the present study we investigated the situation in a particular high-prevalence community. However the methods could be applied to low-burden settings as well, where infectious people are heterogeneously distributed. Within particular transmission hot spots such as prisons, homeless shelters, or immigrant clusters, prevalences of >500 cases per 100,000 people would suggest that transmission elasticity becomes relevant. If averaged over the entire low-burden setting, the effect may not be observable.

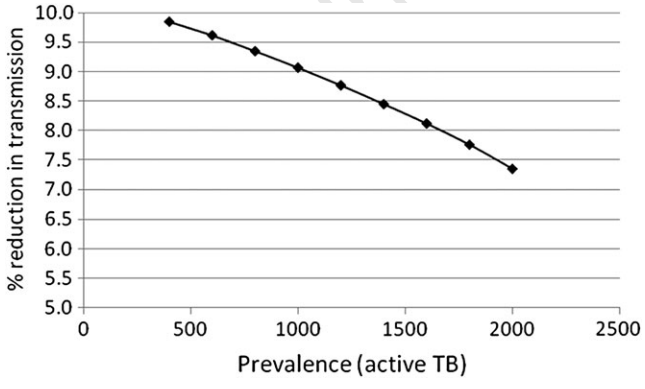

Figure 4. Effect of a 10% reduction in tuberculosis (TB) prevalence on annual infection risk within the total population at various tuberculosis prevalence rates. The proportion of the community considered part of the high-risk component is 39% [12].

DISCUSSION

In an environment where tuberculosis is hyperendemic, it is likely that susceptible individuals may be simultaneously exposed to >1 infectious person with tuberculosis. Previous mathematical models failed to consider the potential importance of this transmission overlap, which has particular relevance in hyperendemic communities. Reducing the tuberculosis prevalence will reduce ongoing transmission, but our data suggest that this reduction is highly variable (transmission elasticity) depending on the initial prevalence rate and likelihood of transmission overlap. Transmission elasticity may partly explain why the transmission impact observed in some communities that achieved and maintained global performance targets failed to meet expectations. Although the effect seems modest at an averaged population level, it is likely that transmission elasticity is greatly enhanced within particular transmission hot spots where high-risk individuals are likely to congregate.

Only 2 avenues exist to gain control of the global tuberculosis epidemic: (1) reduce host vulnerability at the population level and/or (2) limit ongoing *M. tuberculosis* transmission. The challenge is greatly increased by factors such as the coexistent human immunodeficiency virus (HIV) infection epidemic,

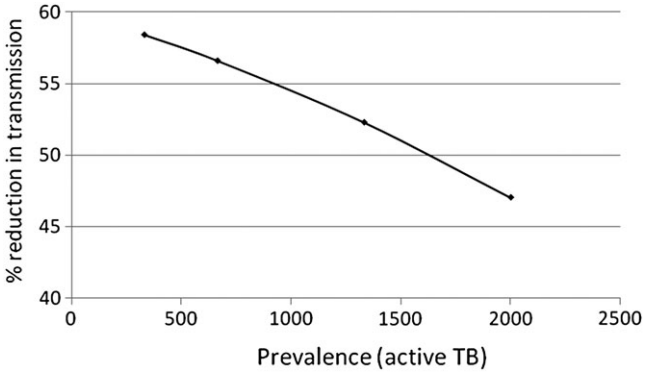

Figure 5. Percentage decrease in annual risk of infection with a 60% reduction in tuberculosis (TB) prevalence at different case densities (initial prevalence rates).

drug-resistant tuberculosis, and the multiple social determinants of tuberculosis [4, 5]. Our work focused exclusively on transmission dynamics and the development of mathematical models that are more robust and predictive in hyperendemic areas. Although we utilized data from a particular setting, we believe the underlying principles are generalizable and transmission elasticity should be considered in future attempts to model tuberculosis epidemiology in hyperendemic settings. Simplified models are likely to overestimate the epidemiological impact that will be achieved in areas with significant transmission overlap, as is likely to occur within transmission hot spots. A remaining shortcoming of current transmission models is their failure to consider the impact of diagnostic delay and transmission saturation [20]. With delayed diagnosis, the transmission impact of curative treatment is greatly reduced [21]. Current targets do not reflect the importance of limiting diagnostic delay, which is essential to reduce ongoing transmission.

Transmission elasticity demonstrates that any control policy is likely to be less effective than predicted by current models especially in high-prevalence settings. Dowdy and Chaisson [22] showed that once case detection rates stabilize at any constant level $<80\%$, no further reduction in tuberculosis incidence occurs. Adding the transmission elasticity effect to this finding provides further support for efforts to optimize case detection and treatment success, irrespective of whether existing targets have been met. Delays in accurate diagnosis and institution of effective treatment erode the effectiveness of target interventions, which has particular relevance for the spread of drug-resistant tuberculosis [23, 24].

In conclusion, the incorporation of transmission elasticity in future mathematical models supports the identification of more stringent performance targets for global tuberculosis control. Tuberculosis control programs should vigorously pursue improvements in case detection, reductions in diagnostic delay, and reductions in time to effective treatment, particularly in hyperendemic areas where transmission elasticity is likely to be most relevant.

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Potential conflicts of interest. All authors: No reported conflicts.

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Rates of Tuberculosis Transmission to Children and Adolescents in a Community with a High Prevalence of HIV Infection among Adults

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(See the editorial commentary by Rieder on pages 356–7)

Background. Tuberculin skin test surveys are routinely used to test for tuberculosis (TB) infection in communities, but there are few data from tuberculin skin test surveys from countries in which both TB and human immunodeficiency virus (HIV) infection are prevalent.

Methods. We conducted a tuberculin skin test survey among 831 school-going children aged 5–17 years in a community that was experiencing an increase in the prevalence of TB and HIV infection. Responses to purified protein derivative RT23 were measured 3 days after the test was administered to determine tuberculin skin test results.

Results. The prevalence of tuberculin skin test results positive for TB (i.e., an induration $\geqslant$10 mm in diameter in response to the skin test) ranged from 26.2% among children aged 5–8 years to 52.5% among children aged 14–17 years. The overall annual risk of infection was 4.1% using a 10-mm cutoff and 2.0% using a 17.4-mm cutoff. Annual risks of infection were constant across age groups. This is consistent with the finding that TB incidence remained the same in children ($P = .48$) from 1999 through 2005, although total TB incidence and adult TB (determined by sputum smear test) incidence increased in this community during the same period ($P < .001$).

Conclusions. The annual risk of infection is high in the community. It appears that HIV infection–associated TB is not a major influence on the annual risk of infection and that TB transmission from adults to children may be associated with a subset of TB cases in the community. An improved understanding of TB transmission patterns is urgently needed help the implementation of novel strategies for reducing the annual risk of infection in this setting.

The directly observed therapy short-course strategies that are currently recommended by the World Health Organization are insufficient to contain the tuberculosis (TB) epidemic in countries with a high prevalence of HIV infection [1–3]. It is unclear how the HIV infection–related TB epidemics impact the children in affected communities. The tuberculin skin test (TST) is an epidemiological tool to test for TB; because the prevalence of TB among young children may be interpreted as incidence, data generated by TST surveys can be used to calculate an annual risk of TB infection (ARTI). Although limitations attributable to the methodological weaknesses inherent in this technique are recognized [4, 5], TST surveys are valuable for the detection of TB in communities with low case-detection rates and for assessing the impact of HIV infection on a TB epidemic [6]. Additionally, TST surveys have an important role in understanding the impact of both HIV infection and TB epidemics on children.

In the prechemotherapy era, Styblo [7] described a fixed relationship between ARTI and disease incidence (based on sputum smear results positive for TB). This theory has been criticized [5], because the advent of

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TB chemotherapy would alter any relationship between ARTI and TB incidence [4, 5, 8, 9]. TB treatment increases survival rates and reduces person-time of infectiousness in a community, thereby reducing infection risk [4] and subsequently decreasing TB prevalence and incidence. However, these factors are all dependent on the performance of the TB control program [4, 5, 8], which may vary over time and between different communities. The relationship between ARTI and TB incidence may have been altered by the impact of the HIV epidemic on TB. This change is postulated on the basis of the increase in the number of TB cases with both positive and negative sputum smear results in areas with a high prevalence of HIV infection and the changes in the duration of infectiousness of HIV-infected TB patients [4, 10]. However, there is conflicting evidence regarding whether HIV-infected TB patients are as infectious as non–HIV-infected TB patients [11–13].

There have been few studies regarding the impact of the HIV epidemic on TB infection among children, and available findings are mixed. One study from Tanzania reported a significant decrease in ARTI among children aged 6–14 years for the period 1983–2003 [14] in the context of an increasing HIV epidemic (HIV infection prevalence, 7%–11% [15]). This trend was reported despite an increase in TB notification rates [14]. In 1994, a study of Ugandan children reported a decrease in ARTI among school-going children [16]. In contrast, research from Kenya reported an increased ARTI during the period 1986–1996, which was associated with an increasing TB epidemic in areas with a high prevalence of HIV infection [17]. These surveys have reported ARTIs ranging from 0.68% to 1.2% [14, 16, 17]; the variability in ARTI may be attributable to the differing performance of TB control programs in the study communities. There is little recent published tuberculin skin test data from southern Africa, where the prevalence of HIV infection has reached unprecedented levels [18].

We have previously reported on the growing TB epidemic in a periurban township that is heavily affected by HIV infection in Cape Town, South Africa. The prevalence of HIV infection among adults in this community is 23% [19], and it is 10% among adolescents aged 11–19 years [20]. The National TB Control Program, which operates on the basis of World Health Organization directly observed therapy short-course recommendations, is well run, with treatment completion and cure rates of patients with positive sputum smear results of ~80% [21] and case-finding rates of 67% among non–HIV-infected patients with positive sputum smear results, but case-finding rates are lower among HIV-infected patients with positive sputum smear results [19]. However, local TB notification data shows a rapid increase in TB notification rates among adults, from 789 notifications per 100,000 population in 1996 to >1900 per 100,000 population in 2005, with the incidence rates of TB with positive sputum smear results among adults increasing from 326 per 100,000 population in 1996 to 1307 per 100,000 population in 2005 [3, 19]. A cross-sectional survey of TB prevalence performed in 2005 showed an overall prevalence of TB with positive sputum smear results of 1495 per 100,000 population, and 0.8% of these cases were untreated [19]. We believe that, in this community that has an adequate TB control program, the HIV epidemic is driving the increase in TB incidence [3]. To better understand patterns in the transmission of TB in this context, we assessed the prevalence of TB infection among children aged 5–17 years in this setting of increasing TB and HIV infection prevalence.

METHODS

The TST survey was performed on a sample of children attending school in the community, which is comprised of ~13,000 predominantly Xhosa-speaking individuals who live in a very poor, high-density residential area. A cross-sectional survey was performed in 2 stages among school children attending the local government primary school. Children were eligible if they were a resident in the community and registered at the local school. Children in grades 1–3 were enrolled in October 2006 and children in grades 5–7 were enrolled in October 2007. Parental consent and assent from participants who were >6 years of age was obtained prior to enrollment. The survey was performed on the school premises. Basic demographic information was collected for each participant. All participants were examined for the presence of a bacille Calmette−Guérin (BCG) scar; the TST was performed for participants regardless of BCG scar status. The World Health Organization–recommended standard of 2 TU of purified protein derivative RT23 with polysorbate 80 (Tween 80, Statens Seruminstitut) was administered intradermally to the volar surface of the left forearm by a trained nurse.

The size of the reaction to the tuberculin was measured by a trained reader at a second visit 3 days after the inoculation. The presence or absence of a reaction was noted, and, where present, the size of the induration was measured along perpendicular axes using standard calipers.

This study was approved by the University of Cape Town's Research Ethics Committee, and all procedures were in accordance with the ethical standards of this committee and the Declaration of Helsinki, 1975, as revised in 1983. All children with a TST reaction induration ⩾10 mm were recalled for investigation for active TB, and children with signs or symptoms of active disease were referred to the local clinic for additional management.

Data were analyzed using STATA, version 9.0 (StataCorp). Results reported here were calculated as the mean of the 2 diameters of the TST reaction induration: a positive reaction was defined at 10-mm and 17.4-mm cutoff points in separate analyses. The 10-mm cutoff was based on guidelines for in-

Table 1. Demographic characteristics of study participants.

Characteristic	Age group				All (n = 831)	P
	5–8 years (n = 233)	9–11 years (n = 222)	12–13 years (n = 237)	14–17 years (n = 139)		
Mean age, years	7.3	9.9	12.5	14.6	10.7	
Sex						
Male	115 (49)	122 (55)	110 (46)	87 (63)	434 (52)	.01
Female	118 (51)	100 (45)	127 (54)	52 (37)	397 (48)	
BCG scar status						
BCG scar observable	30 (13)	66 (30)[a]	87 (37)	30 (22)	213 (25)	<.001
No BCG scar observable	203 (87)	155 (70)[a]	150 (63)	109 (78)	617 (75)	

NOTE. Data are no. (%) of patients, unless otherwise indicated. BCG, bacille Calmette Guérin.

[a] One participant's BCG scar status was indeterminable because of burn scars (n = 221).

fection in clinical settings [22]; the 17.4-mm cutoff was determined as the mean induration size, excluding all nonreactive individuals [23]. ARTI was calculated as follows:

$$1 - (1 - \text{prevalence})^{1/\text{mean age}+0.5}.$$

Because the age (in full years) of the patient at their most-recent birthday was used, 0.5 was added to the mean age for the calculation of ARTI [23]. The cohort was divided into groups of participants aged 5–8 years, 9–11 years, 12–14 years, and 15–17 years, and prevalence and ARTI were calculated overall and for each age group. Bivariate analyses employed the Student's t test and Fisher's exact test, as appropriate. Wilcoxon rank-sum tests were used for the comparison of TST results between different groups. Multiple logistic regression models were developed to examine factors associated with positive TST results. A χ^2 test for trend was used to assay for a trend between age and ARTI, as well as for changes in TB notification rates over the 5-year period. All statistical tests were 2-sided, with $\alpha = 0.05$.

The number of notified TB cases in children was obtained from the TB register at the community TB clinic, and TB prevalence rates were calculated using the 1996 South African National Census and the 2003, 2004, and 2006 house-to-house census performed by the Desmond Tutu HIV Centre. Ratios of TB prevalence and incidence of new TB infections were calculated from the ARTI and from TB prevalence and incidence data already published for this community [3, 19].

RESULTS

Of the 1060 children enrolled in the target grades at the primary school, 1020 (96%) were eligible for study participation. Of the 40 children excluded from the study, 18 (45%) were ineligible because they lived outside of the community, and 22 (55%) were ineligible because they had dropped out of school during the course of the year. Consent was obtained from the parents of 837 (82%) of the eligible children. The parents of 44 children (4%) refused consent and 137 children (13%) had incorrect location information listed in the school register. We enrolled 832 children in the study (99% of children from whom consent was obtained). The outstanding 5 children were not enrolled because of persistent absenteeism. Tuberculin reaction was assessed in all children who were inoculated, except 1, who relocated before the reaction was assessed. Thus, this child was excluded from the analysis (n = 831).

Table 1 shows the demographic characteristics of the study cohort. Ages ranged from 5 to 17 years with a mean age of 10.7 years, and 52% of the participants were male. The majority of the children (74%) did not have a BCG scar; 1 child's BCG scar status was unobservable because of burn scars on the upper arm, and the child was thus excluded from analysis involving BCG scar status. In the crude analysis, older children (children of age greater than the mean) were more likely to have BCG scars than were children aged less than the mean age (P< .001).

The frequency distribution of reaction induration sizes for positive TST results is presented in figure 1. There were no significant differences between the mean reaction induration sizes of participants who had BCG scars and the mean reaction induration sizes of those who did not have scars (6.2 vs. 7.0 mm, P = .28).

The cutoff point, determined by the mean induration size excluding TST nonreactors, was 17.4 mm. Using the 10-mm cutoff, 311 participants (37%) had a TST result positive for TB; using the 17.4-mm cutoff, 171 participants (21%) had a result positive for TB. At both the 10-mm and 17.4-mm cutoff points, there was no significant difference in the defined TB positivity by sex (P = .97 and P = .69, respectively) or BCG status (P = .39 and P = .08, respectively). In a logistic regression model that predicted the relative odds of a positive TST result, age was positively associated with a positive TST result using both the 10-mm cutoff (adjusted OR for a 1-year increase

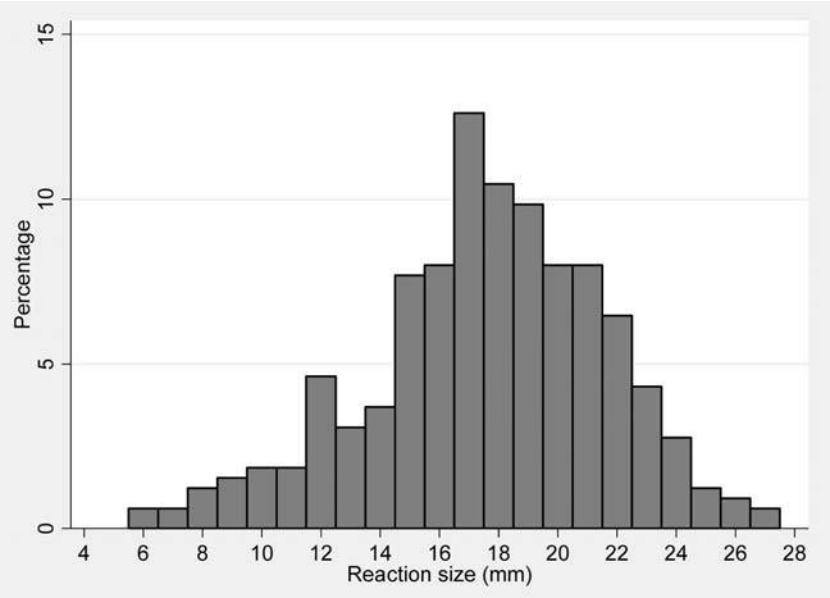

Figure 1. Frequency distribution of positive tuberculin skin test reactions.

in age, 1.19; 95% CI, 1.12– 1.25; $P < .001$) and the 17.4-mm cutoff (adjusted OR, 1.19; 95% CI, 1.12– 1.27; $P < .001$).

The total ARTI for this population was 4.1% using the 10-mm cutoff and 2.0% using the 17.4-mm cutoff. The ARTI did not differ significantly across the age quartiles for the 10-mm or the 17.4-mm cutoff ($P = .50$ and $P = .39$, respectively). Tables 2 and 3 report TB prevalence and ARTI by age quartiles for both cutoffs.

TB notification rates among children ranged from 456 notifications per 100,000 population in 1999 to 395 per 100,000 population in 2005. Figure 2 shows the change in notification rates of total cases of TB, cases of pulmonary TB with positive sputum smear results in adults, and cases of TB in children in this community for the period 1999–2005. The rate of total cases of TB in the population and the rate of TB with positive sputum smear results in adults has increased significantly over this period ($P < .001$ for both, by test for trend). However, TB notification rates in children have not changed significantly during the same period ($P = .48$, by test for trend).

The ARTI of 4.1% indicates that there should be 4100 exposures per 100,000 population each year. On the basis of the incidence of new TB infections with positive sputum smear results in 2005 (1459 cases per 100,000 population) [19], we estimated that the ratio of TB incidence to new infections is approximately 1:2.8. Because 0.8% of TB cases with positive sputum smear results in the community are untreated [19], the ratio of prevalence to new infections is estimated to be 1:5.

DISCUSSION

To our knowledge, this is the first study from southern Africa reporting ARTI in the context of a rapidly growing epidemic of TB and HIV. ARTI is a measure of TB infection risk for previously unexposed individuals. A fixed relationship between the prevalence of TB (as a measure of ARTI) and the incidence of TB with positive sputum smear results was proposed in the prechemotherapy era [8], although this relationship has been increasingly questioned [5]. It is recognized that chemotherapy, TB case-finding, and the performance of the TB control program also affect ARTI by modifying the person-time of infectiousness in the community [4, 5, 8, 9]. The HIV epidemic has a profound influence on the epidemiology of TB. However, individuals who have TB who are coinfected with HIV may be less likely to infect their close contacts than are non–HIV-infected individuals who have TB [11, 12]. The relationship between ARTI and HIV infection–associated TB at a population level is therefore unclear. This study was performed in a well-defined community with detailed information available regarding TB incidence and prevalence and TB control program performance parameters, together with longitudinal data on HIV and HIV-TB coinfection prevalence.

The main finding of the study is that, regardless of the cutoff used for positivity, the ARTI observed in this study was markedly higher than those documented in other sub-Saharan countries that were heavily affected by HIV infection (2.0% or 4.1% in our study vs. 0.68%–1.2% in other studies [14, 16, 17]). The prevalence of latent TB infection of 70%–83% among young, non–HIV-infected adult controls from similar communities in Cape Town is consistent with this high ARTI rate [24, 25]. With no TST induration size results between 0–5 mm and little evidence of the cross-reaction with environmental *Mycobacterium* species that was present in other studies, the high prevalence of TB infection allowed us a unique opportunity to accurately

Table 2. Tuberculosis prevalence and annual risk of tuberculosis infection (ARTI) using the 10-mm cutoff for tuberculin skin test (TST) induration size, by age quartile.

Age category, years	Mean age, years[a]	No. of participants	No. of participants with positive TST result	Prevalence, %	ARTI, %
5–8	7.8	233	61	26.2	3.8
9–11	10.4	222	70	31.5	3.6
12–13	13.0	237	107	45.1	4.5
14–17	15.1	139	73	52.5	4.8
All	11.2	831	311	37.4	4.1

[a] The mean age reported here is the age used for the ARTI calculations and was determined by adding 0.5 years to the mean age for the age category.

assess ARTI by age strata, providing a measure of prevalence over time. Surprisingly, we found that the ARTI remained constant across the age range of 5–17 years, despite the fact that children were being exposed to a rapidly escalating TB epidemic. This finding is additionally supported by the fact that the TB notification rate among children <14 years of age remained unchanged during the past 7 years; however, it should be noted that, because TB case detection rates are low in this community, there may be more cases of TB among children than were detected by the TB control program.

We have shown previously that HIV infection is driving the increasing TB epidemic, especially among adults aged 20–40 years in this community, an age group with the highest prevalence of HIV infection [3]. However, ARTI has remained stable during a period when the prevalence of HIV infection among adults has increased from 14% to 23% [3, 19]. In 2005, >70% of patients with TB were also HIV infected, and TB notification rates among HIV-infected patients were nearly 7-fold greater than those among non–HIV-infected patients [3]. Therefore, it appears that HIV infection–associated TB is not the major determinant of the ARTI in this community. This would be consistent with the findings from other studies of lower infectivity of HIV-infected TB patients [11, 12]. It may also reflect that children are not proportionately affected by the HIV epidemic, because their predominant social contacts are peers, parents, and teachers.

Despite a significant increase in the total number of pulmonary TB cases and in the number of pulmonary TB cases with positive sputum smear results among adults in this community during the past 5 years, the TB incidence among children has remained stable. The ratio of ARTI to the number of new TB cases with positive sputum smear results decreased from 1:6 in 1999 to 1:3 in 2006. However, the more relevant ratio between the ARTI and the cases of untreated TB with positive sputum smear results was only 1:5 in 2005. These data are based on a small study sample performed at a single school in one community, and therefore, they may not be generalizable to other regions.

Since 1960, the policy in South Africa has been to vaccinate all infants with the BCG vaccine [26]. However, only 25% of the children in this study had an observable BCG scar, although BCG scarring may be variable [27, 28]. We also found no difference in the distribution of TST results between those children with and those children without BCG scars, and we therefore included all children in the analysis. This lack of correlation between the presence of a BCG scar and a tuberculin reaction has been shown in other studies [28–30].

Tests for HIV infection were not performed in this survey,

Table 3. Tuberculosis prevalence and annual risk of tuberculosis infection (ARTI) using the 17.4-mm cutoff for tuberculin skin test (TST) induration size, by age quartile.

Age category, years	Mean age, years[a]	No. of participants	No. of participants with positive TST result	Prevalence, %	ARTI, %
5–8	7.8	233	25	10.7	1.4
9–11	10.4	222	39	17.6	1.8
12–13	13.0	237	71	30.0	2.7
14–17	15.1	139	36	25.9	2.0
All	11.2	831	171	20.6	2.0

[a] The mean age reported here is the age used for the ARTI calculations and was determined by adding 0.5 years to the mean age for the age category.

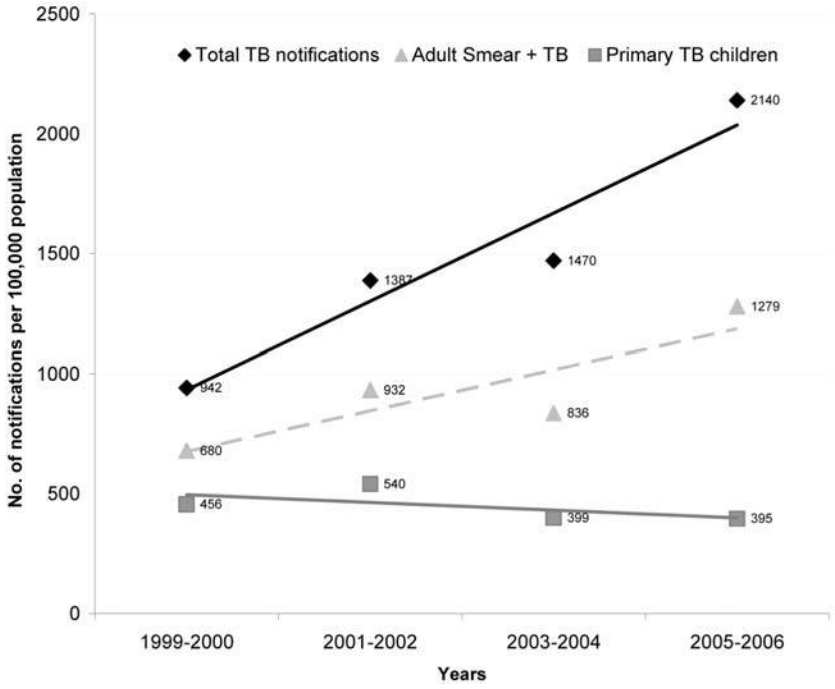

Figure 2. Changes in notification rates of total tuberculosis (TB) cases, cases of pumonary TB with positive sputum smear results in adults, and cases of TB in children (aged <14 years) in the study community during the period 1999–2005.

because this might have adversely affected parental willingness to consent to their child's participation. Study participants were born in the period 1990–2001, when the reported HIV infection prevalence at antenatal clinics ranged from 0.7% to 24% [31]. This was prior to the introduction of the prevention-of-mother-to-child-transmission program. With a transmission rate of ~30%, we estimate that 0.2% of the older cohort and up to 7% of the younger children may have been infected with HIV perinatally. However, considering the high early mortality rate among HIV-infected children [32], it is probable that a maximum of 0.1%–4% of children in this study sample were HIV-infected. This is a small percentage and is unlikely to have had a large impact on the TB prevalence. However, any impact of HIV infection on the prevalence of TB would have also been offset by the skin test anergy caused by advanced HIV disease, which results in false-negative TST reactions. Additionally, it is possible that there is a bias among school attendees and that non–school-attending children may have higher TB infection rates; this may have resulted in an underestimation of the TB prevalence.

An ARTI of 2.0%–4.1% would result in the majority of individuals being latently infected before reaching adulthood, and would ensure the continued exposure of these individuals to further TB infection. The combination of high latent infection rates, repeated ongoing exposures, and an extremely high lifetime risk of HIV infection may go a long way towards explaining the explosive epidemics of HIV infection and TB [3]. TB control will require measures that are targeted at decreasing the ARTI. The current TB control program implements a directly observed therapy short-course policy and reports treatment completion rates >80%. The finding rate of TB cases with positive sputum smear results in this community is below World Health Organization targets and could be improved by active case finding among both non–HIV-infected and HIV-infected individuals. Preventative isoniazid therapy is not routinely used for either TB control or HIV programs, and its role needs to be explored. An antiretroviral treatment program has been instituted, and >30% of HIV-infected individuals are receiving therapy (data not shown). Although antiretroviral therapy will likely decrease TB rates among HIV-infected individuals, the findings of this study suggest that this measure is unlikely to positively impact the ARTI. This is possibly because this measure may provide limited information about the epidemic among population groups that are not primarily composed of the participants' parents and teachers. In this poor community, the impact of social policies that are intended to improve housing and decrease crowding also cannot be ignored.

This study shows high ARTI rates among school-going children in this setting of rapidly growing TB and HIV epidemics. The ARTI observed was markedly higher than those published for other sub-Saharan countries that are similarly affected by HIV infection. The ARTI was constant across the age groups studied, which indicates that the HIV infection–driven TB epidemic has not directly affected children in this community.

Novel strategies for reducing the ARTI need to be considered, and this study highlights the importance of improving the understanding of TB transmission in this setting.

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Mathematical Biology

Modeling the joint epidemics of TB and HIV in a South African township

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Abstract We present a simple mathematical model with six compartments for the interaction between HIV and TB epidemics. Using data from a township near Cape Town, South Africa, where the prevalence of HIV is above 20% and where the TB notification rate is close to 2,000 per 100,000 per year, we estimate some of the model parameters and study how various control measures might change the course of these epidemics. Condom promotion, increased TB detection and TB preventive therapy have a clear positive effect. The impact of antiretroviral therapy on the incidence of HIV is unclear and depends on the extent to which it reduces sexual transmission. However, our analysis suggests that it will greatly reduce the TB notification rate.

Keywords HIV · TB · Epidemic model · Bifurcation diagram

Mathematics Subject Classification (2000) 34C60 · 92D30

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1 Introduction

In South Africa, 5.5 million people are infected with the human immunodeficiency virus (HIV), that is 12% of the country's total population [66, p. 455]. Approximately 270,000 cases of active tuberculosis (TB) are notified each year [76, p. 137]. Among adult cases of active TB, nearly 60% are HIV_+ because coinfection with HIV and *Mycobacterium tuberculosis* (MTB) increases greatly the probability of progressing from latent to active TB.

Detailed studies of these epidemics in a township near Cape Town have been published recently [35,75]. Estimates of the TB notification rate (based on the yearly number of TB notifications, on two population censes conducted in 1996 and in 2004, and assuming a linear population increase in between) and of the prevalence of HIV (estimated using data from an antenatal clinic) are shown in Table 1.

For the year 2005, 259 TB cases were reported among adults (age $\geq$ 15) [75]; 66% of those who were tested for HIV were HIV_+. The adult population was then estimated to be 10,400 and the total population 13,000. So the TB notification rate in the whole population was over $259/13,000 \simeq 1,992$ per 100,000 per year. Moreover, in a sample population of 762 adults, 12 had undiagnosed TB (3 HIV_- and 9 HIV_+). Around 23% (174/762) of the sample population was HIV_+. More than 80% of smear-positive TB cases receiving treatment were cured.

There have been many studies in the medical literature focusing on particular aspects of the joint HIV–TB epidemics in this and other similar townships near Cape Town [3,34,35,37–40,75]. In the present paper, we build a mathematical model to integrate the data on TB and HIV in order to develop a better understanding of the epidemic. We keep the model as simple as possible consistent with the available data and we do not stratify the model by age. The main focus is on the impact of various control measures. Given the extremely high levels of both HIV and TB in this setting, it is essential to know what are the most effective control measures. Of particular importance is the fact that a substantial project is being planned to control HIV and TB in this township. The model may help the planning and design of the intervention. Furthermore, the model and its predictions may provide a framework for evaluating the success or failure of the intervention.

Section 2 reviews mathematical models that have previously been developed to investigate joint epidemics of HIV and TB. Section 3 introduces the model we use, which we have tried to keep as simple as possible. Section 4 analyzes some mathematical properties of the model. Section 5 reviews parameter values in the medical literature. Section 6 estimates several parameters using the data from the South African

Table 1 TB notifications per 100,000 per year and HIV prevalence (%)

Year	1996	1997	1998	1999	2000	2001	2002	2003	2004
TB	580	653	913	897	982	1,410	1,366	1,472	1,468
HIV	6.3	8.9	11.6	14.2	16.5	18.4	19.9	21.1	21.9

Data from [35, Table 1]

township. Section 7 contains bifurcation diagrams showing qualitatively and quantitatively how the steady states of the model change for different sets of parameter values. This approach is needed since some parameters are known only approximately. Section 8 investigates how various control measures might affect the HIV and TB epidemics with a focus on transient dynamics, since the convergence to a steady state takes many decades. The main question is about the impact of antiretroviral therapy (ART) on the TB notification rate, the answer to which is not obvious. Indeed, coinfected people on ART have a risk of developing TB reduced by 80%, but their life expectancy is also greatly increased. As their risk of developing TB is still several times higher than for HIV_- people, this may increase TB transmission. Our numerical results suggest the contrary: ART could decrease considerably the TB notification rate even as it increases the prevalence of HIV. This conclusion should be considered with caution as there are uncertainties not only in parameter values but also in model formulation.

2 Review of HIV–TB epidemic models

Table 2 reviews HIV–TB epidemic models. The models have been of essentially two different types: either computer simulation studies focusing on transient behavior of realistic but complex models, or "mathematical" studies of simpler but less realistic models focusing on steady states and their stability. These models have considered the situation in sub-Saharan Africa, the USA, Russia, India, or in Brazilian prisons. Some models tried to present a global view by considering all of the five WHO-regions. Other models did not focus on any specific area. The compartments combined a certain number of HIV-states (call it i) and a possibly different number of TB-states (call it j). In such a case, one would expect the model to contain $i \times j$ compartments. Some models have aggregated several compartments while others have added more compartments to take into account specific interventions. This is why the number of compartments is written as $i \times j \pm k$ in Table 2. Some models took the form of a system of ordinary differential equations (ODEs). Most others used discrete-time difference equations. Finally, we mention the ongoing work of Lungu [43]. Several other models have considered generically two diseases infecting a single population, but either they did not include a separate compartment for coinfected people [47], or they did not include a latent state [5], an important feature of TB.

All these models contain many unknown parameters but rely on little data. For example, it seems that [14,15,32,57,70] were the only ones to fit their parameters by using real time series of both HIV prevalence (AIDS cases in [70]) and TB notifications. For the South African township under study, we have two extra pieces of information: the percentage of HIV_+ people among TB notifications and the prevalence of TB at one time point. These two extra constraints should make our parameter estimations more robust. Moreover, the township is certainly more homogeneous than whole countries (the USA in [70], Kenya in [14,15], Zimbabwe in [32]) and less exceptional than a female prison [57]. Besides, we have focused our attention on one of the simplest models we could reasonably think of, with a minimum number of compartments and parameters but even so, our model contains 22 parameters. This should also make our estimates more robust.

Table 2 Review of HIV–TB models

Year	References	Type of model, area studied, model structure and summary
1992	[4]	Static model for sub-Saharan Africa with $2 \times 2 - 2 = 2$ compartments. Affine relationship between TB incidence and HIV prevalence
	[59]	Simulation over 20 years for sub-Saharan Africa (details in [60]). Impact of assumed HIV prevalence increase on TB incidence
1993	[31]	Simulation over 10 years for Uganda with $2 \times 4 = 8$ compartments. TB chemoprophylaxis more efficient than treatment
	[44]	Mathematical analysis of 16 ODEs. Numerical study of the stability of steady states
1994	[60]	Simulation over 20 years for sub-Saharan Africa and Canada structured by age and time since HIV or MTB infection. Impact of assumed HIV prevalence increase on TB incidence
1996	[8]	Simulation over 10 years for the USA with $3 \times 5 - 2 = 13$ compartments, 3 age groups and drug-resistant TB. Combining TB prevention and treatment necessary to reach current goals
1997	[70]	Simulation over 25 years for the USA with 30 ODEs including homosexuals, drug users and immigration. More data on HIV status of TB cases needed
1998	[21]	Simulation over 22 years for the whole World with age structure. Model details no longer on journal website. Impact of WHO TB-strategy on number of deaths
	[51]	Simulation over 32 years for the whole World with $2 \times 19 = 38$ ODEs. Estimation of the size of the TB problem
2000	[17]	Simulation over 30 years for the USA structured by age, sex, ethnicity and location. 14 compartments in TB sub-model
2001	[55]	Stochastic simulation over 2 years for the USA with $5 \times 6 = 30$ compartments. Size of TB outbreaks are very sensitive to TB treatment rate
2002	[56]	Mathematical analysis for Brazil of $3 \times 3 - 1 = 8$ ODEs. Bifurcation diagram of steady states. TB transmission occurs in prisons
2003	[14]	Simulation over 20 years for Kenya, Uganda and South Africa with $3 \times 6 = 18$ compartments. Improving TB detection and treatment more efficient than other interventions
	[57]	Mathematical analysis for Brazil of $3 \times 3 - 2 = 7$ ODEs. Stability of steady states
	[58]	Mathematical analysis of 3 ODES and of a stochastic spatial model for South East Asia. HIV maybe unable to invade populations with high TB burden
2004	[29]	Simulation over 20 years for Uganda of $2 \times 5 + 1 = 11$ ODEs with constant HIV prevalence and BCG vaccination. TB chemoprophylaxis for HIV_+ has a small impact on total TB burden
2005	[1]	Simulation over 20 years for Russia with $3 \times 18 = 54$ compartments. Impact of cure rates for drug-resistant TB on number of deaths
	[72]	Simulation over 40 years for India. Model details not shown. ART necessary to reach Millennium Development Goals for TB
	[15]	Simulation over 20 years for Kenya with $2 \times 6 = 12$ compartments. Improving TB detection and treatment more cost-effective than ART
	[52]	Mathematical analysis of 4 ODEs. Stability of steady states

Table 2 continued

Year	References	Type of model, area studied, model structure and summary.
2006	[10]	Simulation over 30 years for sub-Saharan Africa of $2 \times 22 + 1 = 45$ ODEs. TB chemoprophylaxis speeds up the emergence of drug resistant TB
	[20]	Simulation until steady state for sub-Saharan Africa with $3 \times 8 = 24$ compartments. Impact of better TB diagnostic techniques compared with other interventions
	[32]	Stochastic simulation over 70 years for Zimbabwe with $3 \times 6 = 18$ compartments. 10,000 people in households. Work in progress
2007	[2]	Simulation over 10 years for Russia with $3 \times 18 = 54$ compartments as in [1]. High ART coverage necessary with drug-resistant TB
2008	[63]	Mathematical analysis of $4 \times 4 - 1 = 15$ ODEs with reinfection. Stability of steady states. Backward bifurcation for TB

3 The model

The compartmental structure of our model combines two states for HIV (HIV$_-$ and HIV$_+$) with three states for TB (susceptible, latent TB and active TB as in [46,48, 64]). The notations for the resulting six compartments are shown in Table 3. The subscript 1 always refers to HIV$_-$ people and the subscript 2 to HIV$_+$ people. People in compartments E_1, E_2, I_1 and I_2 are those infected with MTB.

The parameters of the model are shown in Table 4. The physiological parameters are more or less the same for people throughout the world or at least for people living in sub-Saharan Africa: the death rates μ_1 and μ_2, the TB parameters $p_1, p_2, q_1, q_2, a_1, a_2, m_1$ and m_2. On the contrary, the "social" parameters depend on the area under study, in particular on population density and living conditions (the transmission rates k_1 and k_2), access to TB clinics (the detection rates γ_1 and γ_2), quality of treatment (ε_1 and ε_2), sexual habits and local cofactors for the transmission of HIV such as other sexually transmitted diseases and male circumcision (d), speed at which information on HIV diffuses (λ) or epidemic history (t_0). Estimates for most physiological parameters can be found in the medical literature. All "social" parameters have to be estimated from local data.

Table 3 The six compartments of the model and some notations

S_1	Number of HIV$_-$ people who are not infected with MTB
S_2	Number of HIV$_+$ people who are not infected with MTB
E_1	Number of HIV$_-$ people with latent TB
E_2	Number of HIV$_+$ people with latent TB
I_1	Number of HIV$_-$ people with active TB
I_2	Number of HIV$_+$ people with active TB
P	Total population: $P = S_1 + E_1 + I_1 + S_2 + E_2 + I_2$
H	HIV prevalence: $H = (S_2 + E_2 + I_2)/P$

Table 4 The 22 parameters of the model and some extra notations (subscript 1 for HIV$_-$ people, subscript 2 for HIV$_+$ people)

B	Birth rate
μ_1, μ_2	Death rate of people who do not have active TB
k_1, k_2	Maximum transmission rate of MTB
p_1, p_2	Proportion of new infections with fast progression to TB
q_1, q_2	Proportion of reinfections with fast progression to TB
a_1, a_2	Progression rate from latent TB to active TB
β_1, β_2	Recovery rate from active TB without treatment
γ_1, γ_2	Detection rate of active TB cases
$\varepsilon_1, \varepsilon_2$	Probability of successful treatment for detected active TB cases
m_1, m_2	Death rate for active TB cases
d	Maximum transmission rate of HIV
λ	Parameter representing behavior change
t_0	Time of introduction of HIV
p_1', p_2'	Proportion with slow progression to TB: $p_1' = 1 - p_1$, $p_2' = 1 - p_2$
b_1, b_2	Recovery rate from TB: $b_1 = \beta_1 + \gamma_1\,\varepsilon_1$, $b_2 = \beta_2 + \gamma_2\,\varepsilon_2$
$f(H)$	Reduced transmission rate of HIV: $f(H) = d\,e^{-\lambda H}$

The equations of our model are

$$\frac{dS_1}{dt} = B - S_1\,(k_1\,I_1 + k_2\,I_2)/P - \mu_1\,S_1 - f(H)\,H\,S_1\,, \tag{1}$$

$$\frac{dE_1}{dt} = (p_1'\,S_1 - q_1\,E_1)(k_1\,I_1 + k_2\,I_2)/P - (a_1 + \mu_1)\,E_1 + b_1\,I_1 - f(H)\,H\,E_1\,, \tag{2}$$

$$\frac{dI_1}{dt} = (p_1\,S_1 + q_1\,E_1)(k_1\,I_1 + k_2\,I_2)/P - (b_1 + m_1)\,I_1 + a_1\,E_1 - f(H)\,H\,I_1\,, \tag{3}$$

for HIV$_-$ people and

$$\frac{dS_2}{dt} = -S_2\,(k_1\,I_1 + k_2\,I_2)/P - \mu_2\,S_2 + f(H)\,H\,S_1\,, \tag{4}$$

$$\frac{dE_2}{dt} = (p_2'\,S_2 - q_2\,E_2)(k_1\,I_1 + k_2\,I_2)/P - (a_2 + \mu_2)\,E_2 + b_2\,I_2 + f(H)\,H\,E_1\,, \tag{5}$$

$$\frac{dI_2}{dt} = (p_2\,S_2 + q_2\,E_2)(k_1\,I_1 + k_2\,I_2)/P - (b_2 + m_2)I_2 + a_2\,E_2 + f(H)\,H\,I_1\,, \tag{6}$$

for HIV$_+$ people. The flows between the different compartments are shown in Fig. 1.

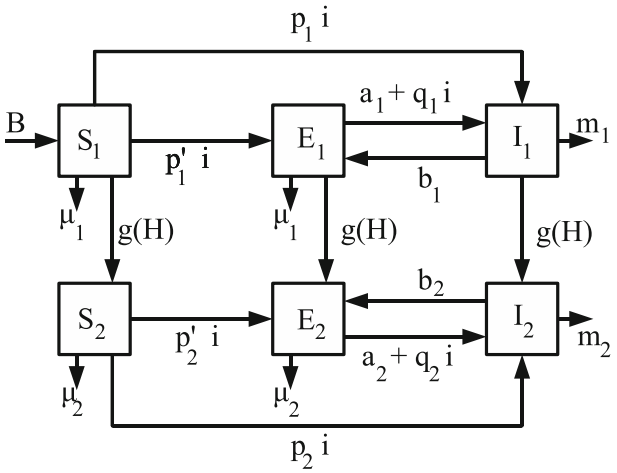

Fig. 1 Flows between the compartments of the model. Here, $i = (k_1 I_1 + k_2 I_2)/P$ and $g(H) = f(H)\,H$

Table 5 Correspondence between some medical vocabulary and the model

TB notification rate	$(\gamma_1 I_1 + \gamma_2 I_2)/P$
MTB infection rate	$(k_1 I_1 + k_2 I_2)/P$
"total" TB incidence rate	$T = a_1 E_1 + a_2 E_2 + (p_1 S_1 + p_2 S_2 + q_1 E_1 + q_2 E_2)(k_1 I_1 + k_2 I_2)/P$
TB incidence rate	T/P
MTB prevalence	$(E_1 + I_1 + E_2 + I_2)/P$
TB prevalence	$(I_1 + I_2)/P$
"Styblo's ratio"	1,000×(TB incidence rate)/(MTB infection rate)
Endogenous reactivation (%)	$(a_1 E_1 + a_2 E_2)/T$
Exogenous reinfection (%)	$(q_1 E_1 + q_2 E_2)(k_1 I_1 + k_2 I_2)/T/P$
Primary disease (%)	$(p_1 S_1 + p_2 S_2)\,(k_1 I_1 + k_2 I_2)/T/P$

Table 5 shows the correspondence we will use between some medical vocabulary and our model. The TB notification rate is the rate at which people in compartments I_1 and I_2 are detected (only a fraction ε_1 or ε_2 of these really move back to the latent compartments E_1 and E_2). The TB incidence rate is the rate at which people enter the compartments I_1 and I_2 divided by the total population usually given "per 100,000 population per year". The MTB infection rate (the continuous-time analogue of the annual risk of infection) is the rate at which people in compartments S_1 (resp. S_2) move to compartments E_1 or I_1 (resp. E_2 or I_2). MTB prevalence is the proportion of the total population in compartments E_1, I_1, E_2 or I_2. TB prevalence is the proportion of the total population in compartments I_1 or I_2. It includes active TB cases, i.e., either undiagnosed TB cases or TB cases that have been detected but that are unsuccessfully treated. We use the expression "Styblo's ratio" to refer to the ratio between TB incidence rate (any form of TB) and MTB infection rate (1,000×). In the literature, the ratio is generally restricted to smear-positive TB notifications (usually about half of all

TB notifications) and the corresponding value has often been assumed to be constant and equal to 50 for HIV$_-$ populations. In other words, an infection rate of 1% per year corresponds to an incidence rate of 50 smear-positive cases per 100,000 per year, or about 100 cases (smear positive and smear-negative) per 100,000 per year. This hypothesis is usually called "Styblo's rule" [6]. However, as we will see in Table 7, Styblo's ratio can no longer be assumed to be the same in areas with a high prevalence of HIV. This remark raises some doubts concerning the method used by Schulzer et al. [59]. Endogenous reactivation is the contribution to the TB incidence coming from compartments E_1 or E_2 at a constant rate a_1 or a_2, exogenous reinfection is the contribution coming from compartments E_1 or E_2 at a rate depending on the number of active TB cases I_1 and I_2. Primary disease is the contribution coming directly from compartments S_1 and S_2 after infection.

A number of key points should be borne in mind:

- At time t_0, we assume that one HIV$_+$ person is introduced in an HIV-free steady population where TB is endemic. We chose this first HIV case to be in state S_2. The formulas for S_1, E_1 and I_1 at the endemic TB steady state will be given in Sect. 4.1.
- Age and sex are not taken into account. In particular, the model cannot distinguish different routes of transmission of HIV, such as sexual transmission and mother-to-child transmission. We did not distinguish pulmonary from extra-pulmonary TB, smear-positive (infectious) TB from smear-negative (non-infectious) TB in order to reduce the number of compartments to a minimum.
- Drug-resistant TB is still very limited in the South African township under study. The efficiency of BCG vaccination is also unclear. We have not included these aspects in our model.
- In Eq. (1), the birth rate is assumed to be a constant independent of the number of people who die of HIV and/or TB. Therefore, our model considers the evolution of cohorts with a fixed size at birth. This is not unreasonable if we use only data on the prevalence of HIV, i.e., the percentage of the population with HIV (not the total number of HIV-infected people), and on the TB notification rate per 100,000 population per year (not the total number of TB notifications during 1 year). If we assumed that deaths are replaced by new "immigrants", we would have to specify their TB and HIV status, something for which it is difficult to get any information. If on the other hand we assumed that births are proportional to the population, then a steady state analysis would become impossible. The demography of the township is in fact quite complex. The population has grown considerably over the past decade. The age pyramid is skewed with more young adults and few children and old people. There are also population inflows and outflows.
- In Eqs. (1) and (4), we chose the "standard form" for TB infection and reinfection as in [24,63,64], and not the "mass action" form used e.g. in [26,46,48]. With a constant birth rate, the total population decreases as the HIV epidemic develops. If we used the "mass action" form for TB transmission, the transmission rate would also decrease and this would artificially slow down the TB epidemic.
- In Eqs. (1)–(3), we also chose the "standard form" for the transmission of HIV as e.g. in [63]. This is the form most commonly used for sexually transmitted

diseases. Following [73] and unlike [63], we assumed however, that the transmission rate is an exponentially decreasing function of HIV prevalence to reflect behavioral changes as HIV awareness develops in the HIV$_-$ population. Reference [73, Suppl.] showed that this special function gives a good fit to HIV infection rate data from another survey in South Africa. It is essential to keep HIV prevalence at realistic levels in a model with no heterogeneity in sexual behavior.

- All other terms are linear. In reality, the rate of progression to active TB is a function of the time since infection, the rate being high during the first 1 or 2 years and relatively low for the rest of one's life [68]. Of course, it is possible to put this into equations [25]. But to keep the number of parameters in the model as small as possible, we have assumed as in [26,46,48,54,64] that a certain fraction of new MTB infections develops active TB immediately, the rest entering a latent state with a constant rate of progression to active TB. Similarly, a certain fraction of reinfections is assumed to lead immediately to active TB as in [24,26,46,48,64]. The other reinfections are "lost" as these people are already latently infected.
- Notice how the equations model people that are unsuccessfully treated for TB. They are counted in the TB notification rate $\gamma_1 I_1 + \gamma_2 I_2$, and induce lower recovery rates $b_1 = \beta_1 + \gamma_1 \varepsilon_1$ and $b_2 = \beta_2 + \gamma_2 \varepsilon_2$ among active TB cases. But they are not counted in a separate compartment.

4 Mathematical analysis

The disease-free steady state with no TB and no HIV is given by $S_1^0 = B/\mu_1$ and $E_1 = I_1 = S_2 = E_2 = I_2 = 0$.

4.1 TB only

Background. The model with TB but no HIV consists only of three compartments (S_1, E_1, I_1) satisfying Eqs. (1)–(3) with $I_2 = 0$, $H = 0$, and $P = S_1 + E_1 + I_1$:

$$\frac{dS_1}{dt} = B - k_1 S_1 I_1/P - \mu_1 S_1 \,, \tag{7}$$

$$\frac{dE_1}{dt} = (p_1' S_1 - q_1 E_1) k_1 I_1/P - (a_1 + \mu_1) E_1 + b_1 I_1 \,, \tag{8}$$

$$\frac{dI_1}{dt} = (p_1 S_1 + q_1 E_1) k_1 I_1/P - (b_1 + m_1) I_1 + a_1 E_1 \,. \tag{9}$$

These equations are up to notations the same as those considered by Singer and Kirschner in [64, Sect. 3]. Building on one side on the earlier work by Feng et al. [24] on a model with four compartments (one more compartment for recovered people) including reinfection but no primary progression (see also the review in [9, Sect. 4.5]) and on the other side on the remarks made by Lipsitch and Murray [42] on the model in [24], reference [64] aimed to show that for a model including all three routes to TB (primary progression, reactivation, and reinfection), a backward bifurcation occurred if the reinfection parameter q_1 was high enough (as noticed in [24]), but too high to be

realistic (as noticed in [42]). In our opinion, there are two weak points in the analysis presented in [64, Sect. 3]. The first point is that, following the idea used in [42], realistic parameters have to satisfy the inequality $q_1 \le p_1$, as latent TB tends to protect against fast progression to active TB in case of reinfection [68]. This inequality did not appear in [64]. The second weak point is that the threshold given in [64, Eq. (7)] is estimated using Latin hypercube sampling of a set of parameter values. With such a method, the conclusion reached is probable but not sure, and can depend on the choice of the set of parameter values. We will show below that the backward bifurcation occurs when q_1 is above a threshold q_1^* which is always bigger than p_1. This proves that the backward bifurcation does not occur for realistic parameter values. Finally, [64] did not show the details of their analysis of the steady states, emphasizing only the conclusions. For our study, we need the formula for the endemic steady state with TB only, as it serves as the initial condition for the full model with both TB and HIV.

One should also mention here the work of Moghadas et al. [46,48] on a model similar to Eqs. (7)–(9) but with "mass action" instead of "standard" incidence. Their model also assumes implicitly that people who have recovered from TB are protected for the rest of their life (they do not return to the latent state), a somewhat unrealistic hypothesis. Formally, this corresponds to the case $b_1 = 0$ in our model. Despite the remarks made by Lipsitch and Murray [42], reference [48] claimed that this backward bifurcation could occur for realistic parameter values. Notice, however, that the parameter values used in [48] for k_1, p_1, and the product $k_1 q_1$ do not satisfy the inequality $q_1 \le p_1$, so they seem to be unrealistic.

Recently, as a part of their analysis of an HIV–TB model, Sharomi et al. [63] studied an extension of the TB-model with four compartments and reinfection introduced by Feng et al. [24]. Again, much emphasis was put on backward bifurcation, which was shown to occur if the ratio q_1/p_1 was above a certain threshold. But this threshold may be bigger than 1 (it is hard to say if this is always so as the formulas for models with four compartments are very complicated). And indeed, the authors chose the unrealistic ratio $q_1/p_1 = 3$ (called η_r in [63]) to illustrate their results.

Analysis. Linearizing system (7)–(9) near the disease-free steady state, we obtain

$$\frac{dE_1}{dt} \simeq k_1 p_1' I_1 - (a_1 + \mu_1) E_1 + b_1 I_1 , \quad \frac{dI_1}{dt} \simeq k_1 p_1 I_1 - (b_1 + m_1) I_1 + a_1 E_1 .$$

So the basic reproduction number R_0^{TB} for TB, as defined in [18], is the spectral radius of the matrix

$$\begin{pmatrix} 0 & k_1 p_1' \\ 0 & k_1 p_1 \end{pmatrix} \begin{pmatrix} a_1 + \mu_1 & -b_1 \\ -a_1 & b_1 + m_1 \end{pmatrix}^{-1},$$

which can easily be computed:

$$R_0^{\mathrm{TB}} = \frac{k_1 (a_1 + p_1 \mu_1)}{a_1 m_1 + m_1 \mu_1 + \mu_1 b_1} . \tag{10}$$

Because this formula does not depend on the reinfection parameter q_1, it is the same as [49, Eq. (10)]. When $b_1 = 0$ and $p_1 = 0$, it is the same as the formula given in [24, Sect. 1]. A slightly more intuitive way of deriving (10) consists in writing that R_0^{TB} is the expected number of secondary infectious cases produced by one infectious index case in an otherwise disease free population. This index case transmits MTB to k_1 people per unit of time and stays infectious on average $1/(b_1 + m_1)$ units of time. Moreover, each new infected person will be immediately infectious with a probability p_1 and infectious only after reactivation with a probability $(1 - p_1)\, a_1/(a_1 + \mu_1)$. Finally, the index case can become infectious again after recovering (possibly several times), with a probability which is the product of $b_1/(b_1 + \mu_1)$ and of $a_1/(a_1 + \mu_1)$. One can check that the formula

$$R_0^{\mathrm{TB}} = \frac{k_1}{b_1 + m_1}\left[p_1 + (1 - p_1)\,\frac{a_1}{a_1 + \mu_1}\right]\sum_{n=0}^{\infty}\left(\frac{b_1}{b_1 + m_1} \times \frac{a_1}{a_1 + \mu_1}\right)^n \tag{11}$$

gives indeed the same result as (10). Since the probability $a_1/(a_1 + \mu_1)$ of developing active TB by reactivation is small, a good approximation for R_0^{TB} would be obtained by replacing the infinite sum in (11) by its first term, which is equal to 1.

Let us look for an endemic TB steady state of the form $(S_1^*, E_1^*, I_1^*, 0, 0, 0)$ of system (1)–(6) with $S_1^* > 0$, $E_1^* > 0$, and $I_1^* > 0$, i.e., a nontrivial steady state (S_1^*, E_1^*, I_1^*) of system (7)–(9). For convenience, let us introduce the following notations:

$$P^* = S_1^* + E_1^* + I_1^*, \quad s_1^* = S_1^*/P^*, \quad e_1^* = E_1^*/P^*, \quad i_1^* = I_1^*/P^*. \tag{12}$$

After some tedious computations, one can show starting from Eqs. (7)–(9) that the fraction of active TB cases i_1^* has to be a positive root of the quadratic equation

$$\begin{aligned}(i_1^*)^2 + \left[\frac{a_1 + b_1 + (1 - p_1)\, m_1 + p_1\mu_1}{q_1 k_1} + \frac{m_1}{k_1} - 1\right] i_1^* \\ + \frac{a_1 m_1 + m_1\mu_1 + \mu_1 b_1}{q_1 k_1^2}\,(1 - R_0^{\mathrm{TB}}) = 0.\end{aligned} \tag{13}$$

Moreover, we have

$$e_1^* = i_1^*\,\frac{k_1 - m_1 - k_1 i_1^*}{\mu_1 + k_1 i_1^*}, \quad S_1^* = \frac{B}{k_1 i_1^* + \mu_1}, \tag{14}$$

from which we can compute

$$s_1^* = 1 - e_1^* - i_1^*, \quad P^* = S_1^*/s_1^*, \quad E_1^* = e_1^* P^*, \quad I_1^* = i_1^* P^*. \tag{15}$$

Quadratic equations similar to Eq. (13) were found in [24, Eq. (A.1)] and [46, Eq. (5.3)]. Set

$$k_1^* = \frac{a_1 m_1 + m_1 \mu_1 + \mu_1 b_1}{a_1 + p_1 \mu_1} . \tag{16}$$

and

$$q_1^* = \frac{a_1 + b_1 + (1 - p_1)\, m_1 + p_1 \mu_1}{b_1 + (1 - p_1)\, m_1} \times \frac{a_1 + p_1 \mu_1}{\mu_1} . \tag{17}$$

Because of (10), we have $R_0^{\mathrm{TB}} = k_1 / k_1^*$. So $R_0^{\mathrm{TB}} < 1$ when $k_1 < k_1^*$, and $R_0^{\mathrm{TB}} > 1$ when $k_1 > k_1^*$. Let us study the steady states of Eqs. (7)–(9) in the parameter space (k_1, q_1). In the appendix, we show that:

- for $q_1 < q_1^*$, system (7)–(9) has no endemic steady state when $0 < k_1 < k_1^*$, and one endemic steady state when $k_1 > k_1^*$ ("transcritical bifurcation" as k_1 increases from 0 to $+\infty$);
- for $q_1 > q_1^*$, there exists another threshold $\widehat{k}_1(q_1) < k_1^*$, depending on q_1, such that system (7)–(9) has no endemic steady state when $0 < k_1 < \widehat{k}_1(q_1)$, two endemic steady states when $\widehat{k}_1(q_1) < k_1 < k_1^*$, and one endemic steady state when $k_1 > k_1^*$ ("backward bifurcation").

Notice that the first fraction in (17) is bigger than 1 and that the second fraction is bigger than p_1. So q_1^* is always bigger than p_1. But realistic values for q_1 are necessarily less than p_1, as already mentioned. This shows that the parameter region with a backward bifurcation is a mathematical curiosity that does not occur in practice, confirming the remarks in [42] and the conclusion suggested by [64]. Notice that formula (17) for q_1^* could have been obtained in [64] if the expression (16) for k_1^* had been inserted in the condition [64, Eq. (7)].

4.2 HIV only

When there is no TB, system (1)–(6) reduces to

$$\frac{dS_1}{dt} = B - \mu_1 S_1 - f(H)\, H\, S_1 , \quad \frac{dS_2}{dt} = -\mu_2 S_2 + f(H)\, H\, S_1 \tag{18}$$

with $H = S_2/(S_1 + S_2)$. Similar epidemic models with a contact rate depending nonlinearly on the number of infected people have been studied for example in [30, 69]. A more complicated model for HIV transmission with a contact rate depending nonlinearly on the prevalence was used in [73]. First, let us linearize the second equation in (18) near the disease-free steady state $S_1 = S_1^0$ and $S_2 = 0$:

$$\frac{dS_2}{dt} \simeq -\mu_2 S_2 + f(0)\, S_2 .$$

Hence, the basic reproduction number for HIV is given by

$$R_0^{\mathrm{HIV}} = f(0)/\mu_2\,.$$

It is easily shown using (18) that any endemic steady state with HIV but no TB has to be given by

$$\widehat{S}_1 = \frac{B\,(1-\widehat{H})}{\mu_1(1-\widehat{H})+\mu_2\,\widehat{H}}, \quad \widehat{S}_2 = \frac{B\,\widehat{H}}{\mu_1\,(1-\widehat{H})+\mu_2\,\widehat{H}},$$

where $\widehat{H}$ is the steady state prevalence of HIV, $\widehat{S}_2/(\widehat{S}_1+\widehat{S}_2)$, and is the solution of the equation

$$(1-\widehat{H})\,f(\widehat{H}) = \mu_2 \tag{19}$$

in the interval (0, 1). Notice that the left side of (19) is a decreasing function of $\widehat{H}$, taking the value $f(0) = d$ when $\widehat{H} = 0$ and the value 0 when $\widehat{H} = 1$. So Eq. (19) has no solution in (0, 1) if $R_0^{\mathrm{HIV}} < 1$ and exactly one solution in (0,1) if $R_0^{\mathrm{HIV}} > 1$.

4.3 HIV and TB

The endemic TB steady state can be invaded by HIV. Linearizing system (4)–(6) near this steady state and using the notations introduced in (12), we obtain

$$\begin{aligned}
\frac{dS_2}{dt} &\simeq -k_1 S_2\, i_1^* - \mu_2\, S_2 + f(0)\, s_1^*\,(S_2+E_2+I_2)\,,\\
\frac{dE_2}{dt} &\simeq k_1\,(p_2'\,S_2 - q_2\,E_2)\,i_1^* - (a_2+\mu_2)\,E_2 + b_2\,I_2 + f(0)\,e_1^*\,(S_2+E_2+I_2)\,,\\
\frac{dI_2}{dt} &\simeq k_1\,(p_2\,S_2 + q_2\,E_2)\,i_1^* - (b_2+m_2)\,I_2 + a_2\,E_2 + f(0)\,i_1^*\,(S_2+E_2+I_2)\,.
\end{aligned}$$

So the basic reproduction number r_0^{HIV} for HIV when introduced in a population at the TB endemic steady state (notice that r_0^{HIV} is different from R_0^{HIV}) is the spectral radius of the matrix

$$f(0)\begin{pmatrix} s_1^* & s_1^* & s_1^* \\ e_1^* & e_1^* & e_1^* \\ i_1^* & i_1^* & i_1^* \end{pmatrix}\begin{pmatrix} k_1 i_1^* + \mu_2 & 0 & 0 \\ -k_1 p_2'\, i_1^* & k_1 q_2\, i_1^* + a_2 + \mu_2 & -b_2 \\ -k_1 p_2\, i_1^* & -k_1 q_2\, i_1^* - a_2 & b_2 + m_2 \end{pmatrix}^{-1}. \tag{20}$$

Notice that this matrix is of rank 1 so the spectral radius is equal to the trace. Hence, one gets

$$r_0^{\mathrm{HIV}} = f(0)\,(s_1^*\,\tau_{S_2} + e_1^*\,\tau_{E_2} + i_1^*\,\tau_{I_2})\,,$$

where τ_{S_2}, τ_{E_2} and τ_{I_2} are complex expressions with a simple interpretation. For example, τ_{S_2} is the life expectation of a person from the moment he/she enters state S_2 (in the linearized model). In particular, τ_{S_2}, τ_{E_2} and τ_{I_2} are all strictly less than $1/\mu_2$ if $m_2 > \mu_2$ (as should be). So

$$r_0^{\mathrm{HIV}} < R_0^{\mathrm{HIV}}.$$

Not surprisingly, the expected number of secondary HIV-cases produced by an "average" HIV_+ person in a population with endemic TB is less then in a population with no TB since active TB may shorten the life of such a person.

Similarly, the endemic steady state with HIV can be invaded by TB. Linearizing Eqs. (2)–(3)–(5)–(6) near $(\widehat{S}_1, 0, 0, \widehat{S}_2, 0, 0)$ and setting

$$\widehat{P} = \widehat{S}_1 + \widehat{S}_2\,, \quad \widehat{s_1} = \widehat{S}_1/\widehat{P} = 1 - \widehat{H}\,, \quad \widehat{s_2} = \widehat{S}_2/\widehat{P} = \widehat{H}\,,$$

we obtain

$$\begin{aligned}
\frac{dE_1}{dt} &\simeq p_1'\widehat{s_1}(k_1 I_1 + k_2 I_2) - (a_1 + \mu_1)\, E_1 + b_1\, I_1 - f(\widehat{H})\,\widehat{H}\, E_1\,,\\
\frac{dI_1}{dt} &\simeq p_1\widehat{s_1}(k_1 I_1 + k_2 I_2) - (b_1 + m_1)\, I_1 + a_1\, E_1 - f(\widehat{H})\,\widehat{H}\, I_1\,,\\
\frac{dE_2}{dt} &\simeq p_2'\widehat{s_2}\,(k_1 I_1 + k_2 I_2) - (a_2 + \mu_2)\, E_2 + b_2\, I_2 + f(\widehat{H})\,\widehat{H}\, E_1\,,\\
\frac{dI_2}{dt} &\simeq p_2\widehat{s_2}\,(k_1 I_1 + k_2 I_2) - (b_2 + m_2) I_2 + a_2\, E_2 + f(\widehat{H})\,\widehat{H}\, I_1\,.
\end{aligned}$$

So the basic reproduction number r_0^{TB} for TB when introduced in a population at the HIV endemic steady state is the spectral radius of the matrix $M\,N^{-1}$, where

$$M = \begin{pmatrix}
0 & p_1' k_1 \widehat{s_1} & 0 & p_1' k_2\, \widehat{s_1}\\
0 & p_1 k_1 \widehat{s_1} & 0 & p_1 k_2\, \widehat{s_1}\\
0 & p_2' k_1 \widehat{s_2} & 0 & p_2' k_2\, \widehat{s_2}\\
0 & p_2\, k_1 \widehat{s_2} & 0 & p_2\, k_2\, \widehat{s_2}
\end{pmatrix} \tag{21}$$

and

$$N = \begin{pmatrix}
a_1 + \mu_1 + f(\widehat{H})\,\widehat{H} & -b_1 & 0 & 0\\
-a_1 & b_1 + m_1 + f(\widehat{H})\,\widehat{H} & 0 & 0\\
-f(\widehat{H})\,\widehat{H} & 0 & a_2 + \mu_2 & -b_2\\
0 & -f(\widehat{H})\,\widehat{H} & -a_2 & b_2 + m_2
\end{pmatrix}.$$

Whether r_0^{TB} is bigger or smaller than R_0^{TB} seems to depend on the numerical values chosen for the parameters.

Assuming realistically that $q_1 \leq p_1$ (so that there is no backward bifurcation for the model with TB but no HIV), this linear stability analysis suggests the following conjecture:

- when $R_0^{\mathrm{HIV}} < 1$ and $R_0^{\mathrm{TB}} < 1$, the disease-free steady state is a global attractor of system (1)–(6);
- when $R_0^{\mathrm{HIV}} > 1$ and $r_0^{\mathrm{TB}} < 1$, the HIV-endemic steady state is a global attractor;
- when $R_0^{\mathrm{TB}} > 1$ and $r_0^{\mathrm{HIV}} < 1$, the TB-endemic steady state is a global attractor;
- in all other cases, there is an endemic steady state with both HIV and TB, which has to be computed numerically, and which is a global attractor.

Since $R_0^{\mathrm{HIV}} > r_0^{\mathrm{HIV}}$, the fourth case contains in fact only two subcases:

- $R_0^{\mathrm{HIV}} > 1$, $r_0^{\mathrm{TB}} > 1$, $R_0^{\mathrm{TB}} > 1$ and $r_0^{\mathrm{HIV}} > 1$. Both the HIV-endemic and the TB-endemic steady states exist but they are saddle points.
- $R_0^{\mathrm{HIV}} > 1$, $r_0^{\mathrm{TB}} > 1$, and $R_0^{\mathrm{TB}} < 1$. The HIV-endemic steady state exists but it is a saddle point. There is no TB-endemic steady state.

5 Parameter values fixed after reviewing the medical literature

5.1 Demographic parameters

Natural mortality was taken to be $\mu_1 = 0.02$ per year as e.g. in [10], corresponding to a life expectancy equal to $1/\mu_1 = 50$ years. This is a little pessimistic even for an area where people live in severe poverty, such as the South African township we are considering. The mortality was assumed to be 0.0064 per year in [31], 0.0081 per year in [29], and 0.0167 per year in [56]. Notice that the mortalities in [29,31] correspond to life expectancies which are much too high.

The birth rate B was chosen to attain a total population for the disease-free steady state ($S_1 = B/\mu_1$) of 10,000, the approximate size of the township [35]. This yields $B = 200$ per year.

5.2 HIV parameters for people not infected with MTB

Mortality for HIV_+ people was taken to be $\mu_2 = 0.1$ per year as is usually done (see e.g. [10]) to get an average survival time of 10 years. This mortality was 0.13 per year in [31] and in [29] (citing a study from Uganda [53]). Schulzer et al. [59] assumed a fixed survival time of 10 years.

5.3 TB parameters for HIV_- people

Parameters p_1 and a_1 modeling the progression to active TB. As already mentioned, the rate of progression to active TB is a decreasing function of the time since infection. Using data from the Netherlands for the period 1951–1970, Sutherland et al. [65] estimated that men have a 5% annual risk of developing primary TB disease during 5 years following the first MTB infection and a 0.025% annual risk of reactivation

after 5 years. For women, the numbers were 6 and 0.002%. Vynnycky and Fine [68] did a similar study using data from England and Wales for the period 1953–1988. For individuals over 20 years old, they estimated that the cumulative risk during the first 5 years was about 14%, with a risk of approximately 8% during the first year, 3% during the second, 1% during the third year. The risk of later reactivation was estimated to be 0.03% per year. For individuals aged 0–10 and 15, the cumulative risks for the first 5 years were 4 and 9% and the risks of reactivation close to 0 and 0.015% per year, respectively. Notice that the cumulative risk during the first 5 years in [65] is about 25%, considerably higher than the 14% from [68]. Our model does not include the time since infection as a variable but assumes instead that a certain fraction of new infections will develop TB immediately while the rest will enter a latent stage where the rate of progression to active TB is constant. Following the more recent estimates of Vynnycky and Fine [68], we will assume that $p_1 = 11\%$ (the estimated cumulative risk for the first 2 years) and $a_1 = 0.03\%$ per year.

Given the natural mortality μ_1 previously chosen, these parameter values correspond to a probability $a_1/(a_1+\mu_1) \simeq 1.5\%$ of progressing from latent to active TB and to a total probability $p_1 + a_1/(a_1+\mu_1) \simeq 12.5\%$ of developing active TB after MTB infection. Notice that it is not sure if parameter estimates of TB progression from a study of British people are relevant for black Africans living in very different conditions. More data is needed on this issue.

As a comparison, the percentage of HIV$_-$ people that progress rapidly to active TB in previous mathematical models was assumed to be 5% in [59] (within 1 year; no reference), 5% in [70] (after a short latent period of about 1 year; no reference), 5% in [29] (immediate progression; no reference), 5% per year in [56] (constant risk; no reference), 7% in [20] (immediate progression; citing [67]), 14% in [10] (after a short latent period of about 1 year; citing [65] and other references), 14% in [32] (within 5 years; citing [65,68] and other references). The rate of reactivation was assumed to be 0.01% per year in [10] (citing [68] and other references), 0.074% per year in [29], 0.1% per year in [20], and 0.1% per year in [32] (after 5 years of infection, also citing [68]). Both [59] and [70] used more complex models taking into account the time since infection. Notice the disagreement concerning parameter values.

Infection versus reinfection: q_1/p_1. Sutherland et al. [65] estimated that a previous MTB infection reduced the risk of disease after reinfection by 63% for HIV$_-$ males and by 81% for HIV$_-$ females. Vynnycky and Fine [68] found a reduction of risk by 16% among HIV$_-$ adolescents and by 41% among HIV$_-$ adults. In their model, Cohen et al. [10] assumed a reduction of risk of 65% for HIV$_-$ people (citing [65,68]). Dowdy et al. [20] assumed a reduction by 72% for HIV$_-$ people and people with early stage HIV (citing [65]). The two previous studies seem to follow the results of [65] rather than the more recent results of [68]. Here, we prefer using an average of the values found in [68]. We assume that $q_1/p_1 = 0.7$, corresponding to a 30% risk reduction for HIV$_-$ people.

Mortality m_1 and natural recovery rate β_1. Data on TB mortality without treatment goes back to the era when no effective treatment was available, that is at the beginning of the twentieth century. The case fatality ratio $[m_1/(m_1+\beta_1)]$ was then approximately

50%. This is the estimate mentioned in the review [50]. Another review [13, Table 1] estimated that the mean duration of disease for untreated HIV$_-$ TB cases $[1/(m_1+\beta_1)]$ was approximately 2 years. These two estimates for $1/(m_1+\beta_1)$ and $m_1/(m_1+\beta_1)$ correspond to $m_1 = 0.25$ per year and $\beta_1 = 0.25$ per year. These are the values that we shall use for our model. Another model assumed 35% deaths after 1 year [31, p. 407]. In those models that considered different mortalities for infectious and non-infectious untreated TB cases, the mortalities were 0.3 and 0.2 per year, respectively [10], or 35 and 10% after 1 year [20]. The rate at which untreated HIV$_-$ TB cases could return to the latent state $[\beta_1]$ was assumed to be 0.2 per year in [10]. All these values are not too far from the ones we have chosen.

5.4 Parameters involving both HIV and TB

The infectiousness ratio k_2/k_1. HIV$_+$ TB cases are on average less infectious than HIV$_-$ TB cases as extrapulmonary TB occurs more often among HIV$_+$ people. Previous models have often split the compartments for active TB cases (whether HIV$_-$ or HIV$_+$) in two, with one sub-compartment for infectious TB and one sub-compartment for non-infectious TB. The percentages of HIV$_-$ and HIV$_+$ TB cases that are infectious were 50 and 40% in [59], 57 and 50% in [29], 45 and 30% in [10]. In the present model, we do not distinguish those TB cases that are infectious from those that are not infectious. Instead, we use an average infectiousness k_1 for all HIV$_-$ TB cases and an average infectiousness k_2 for all HIV$_+$ TB cases. Given the structure of our model, the difference in infectiousness can be taken into account by choosing an appropriate value for the ratio k_2/k_1. Following the numerical values from [10], we assume that $k_2/k_1 = 30/45 = 2/3$.

Progression rate a_2 to active TB for HIV$_+$ people. As for HIV$_-$ people, the rate of progression from latent to active TB depends on the time since infection but also on the stage of HIV infection. However, our model does not distinguish HIV stages, so we will focus only on estimates that are averages over all stages. For HIV$_+$ injecting drug users in the USA, Selwyn et al. [61,62] found an average rate of progression between 0.079 and 0.097 per year. In Cape Town, Badri et al. [3] found an average TB incidence (including reactivation, fast progression, and reinfection) of 0.097 per year. But the incidence of TB was as high as 0.24 per year among HIV$_+$ people in WHO stage 3 or 4 [3]. Following [61,62], we assume for the reactivation rate of our model that $a_2 = 0.08$ per year, an estimate which seems also compatible with the data from [3]. Heymann [31] also used the estimate from [61,62] in his model. Other studies used 0.0074 per year [29] (assuming a ten-fold increase compared to HIV$_-$ people), 0.05 per year [56], or 0.17 per year [10] (no reference). Schulzer et al. [59] used a more complicated model distinguishing whether MTB infection occurred before or after HIV infection. Notice again the disagreement concerning parameter values.

Infection versus reinfection: q_2/p_2. Data concerning reinfection in HIV$_+$ people is scarce. In the outbreak of TB studied by Di Perri et al. [19], none of four individuals that already had a positive tuberculin skin test developed TB. Cohen et al. [10] assumed a

reduction of risk of 25% for HIV_+ people [10, Suppl., Table 2] (no reference). Dowdy et al. [20] assumed a reduction by 25% for people with AIDS (citing [14]). Here, we will assume as in [10] that $q_2/p_2 = 0.75$. But more data is needed to confirm this hypothesis. Recall that for HIV_- people, we assumed that $q_1/p_1 = 0.7$.

Mortality m_2 and natural recovery rate β_2. The mortality of HIV_+ TB cases [m_2] was assumed to be 0.325 per year in [29] (citing [23]) and 1.0 per year in [10] (citing [53]) for both infectious and non-infectious TB. The rate at which untreated HIV_+ TB cases could return to the latent state [β_2] was 0.1 per year in [10]. For our model, we will again use the data from [13, Table 1]: the mean duration of disease for untreated HIV_+ TB cases [$1/(m_2 + \beta_2)$] was given as 0.5 year. In the same reference, the associated case fatality ratio [$m_2/(m_2+\beta_2)$] was 81% for infectious TB (35% of cases) and 76% for non-infectious TB (65% of cases): we use the weighted average, which is close to 80%. These two estimations for $1/(m_2 + \beta_2)$ and $m_2/(m_2 + \beta_2)$ correspond to $m_2 = 1.6$ per year and $\beta_2 = 0.4$ per year.

6 Estimation of the other parameters from the South African data

Proportions ε_1 and ε_2 of successful treatments. The proportion of successful treatments is approximately 80% [75]. We take this value for ε_1 and ε_2.

Detection rates γ_1 and γ_2. [75] reported 259 TB notifications among adults (age $\geq$ 15) in 2005; 66% of those who were tested for HIV were HIV_+. The adult population in that year was estimated to be 10,400. Moreover, in a sample population of 762 adults, 12 had undiagnosed TB (3 HIV_- and 9 HIV_+). So we expect the following equations to hold:

$$\gamma_1 I_1^{\text{adult}} \simeq 34\% \times 259, \quad I_1^{\text{adult}} \simeq 10,400 \times 3/762\,, \tag{22}$$

$$\gamma_2 I_2^{\text{adult}} \simeq 66\% \times 259\,, \quad I_2^{\text{adult}} \simeq 10,400 \times 9/762\,. \tag{23}$$

This gives the estimates $\gamma_1 \simeq 2.2$ per year and $\gamma_2 \simeq 1.4$ per year. But notice that since the ratios 3/762 and 9/762 are small, the uncertainty is large: the 95% binomial confidence interval for the ratios 3/762 and 9/762 are (0.08%, 1.15%) and (0.54%, 2.23%), respectively. Using Eqs. (22)–(23), the corresponding interval for γ_1 is (0.74, 10.6) per year, and the one for γ_2 is (0.74, 3.0) per year. Corbett et al. [12] suggest that γ_2 may be larger than γ_1. For our model, we chose the lower bound of the confidence interval for γ_1 ($\gamma_1 = 0.74$ per year) and the upper bound of the confidence interval for γ_2 ($\gamma_2 = 3.0$ per year). One motivation was that recent unpublished data shows that the MTB infection rate in the past few years has not increased so much. In our simulations, we found that this was only possible with values of γ_2 that are several times higher than γ_1. Indeed, the great increase in TB notifications has to be compensated by a shorter infectious period to keep the MTB infection rate at a relatively low level.

With these choices, we obtain $b_1 = \beta_1 + \gamma_1\varepsilon_1 \simeq 0.84$ per year and $b_2 = \beta_2 + \gamma_2\varepsilon_2 \simeq 2.8$ per year. For comparison, the values used for the whole of Uganda in [29] for b_1

and b_2 were both equal to 0.3 per year, but case detection is probably not as good as in the South African township under study here.

We notice also that the probabilities for TB to be detected are given by

$$\frac{\gamma_1}{m_1+\beta_1+\gamma_1} \simeq 60\%, \quad \frac{\gamma_2}{m_2+\beta_2+\gamma_2} \simeq 60\% .$$

Despite the high death rate m_2, the detection probability for HIV_+ TB cases is the same as for HIV_- because of the high value of γ_2 used here. Recall that the target set by the World Health Organization for case detection is 70%. The average durations of disease are

$$\frac{1}{b_1+m_1} \simeq 0.92 \text{ year}, \quad \frac{1}{b_2+m_2} \simeq 0.23 \text{ year} .$$

As a comparison, Corbett et al. [12] estimated the duration of (smear-positive) disease before diagnosis to be 1.15 and 0.17 year for HIV_- and HIV_+ South African gold miners, respectively.

MTB transmission rate k_1. The average TB notification rate in the decade before 1995 in South Africa, i.e. before the rise of HIV prevalence, was about 200 per 100,000 per year (see [74] and [76, p. 184]). This is also a reasonable estimate for the township under study given the data from Table 1. In our model, the TB notification rate when there is no HIV is $\gamma_1 i_1^*$. Using Eq. (13) for i_1^*, it is possible to estimate the only unknown parameter left: k_1. We take $k_1 = 11.4$ per year, which corresponds to a TB notification rate of 203 per 100,000 per year. In the review [50], each HIV_- person with undiagnosed and untreated smear-positive TB was believed to cause 10 to 14 infections per year. If smear-positive cases make half of all cases, an "average" HIV_- TB case would cause 5–7 infections per year. This range is consistent with our estimate $k_1 = 11.4$ per year for the maximum infection rate in a completely susceptible population and with our estimate of nearly 1 year for the average duration of disease $1/(b_1+m_1)$. If for example $x = 60\%$ of the population is already infected with MTB, one active TB case infects about $x\,k_1/(b_1+m_1)$ susceptible people.

HIV parameters d, λ and t_0. Summing the three equations (1)–(3) for HIV_- people and the three equations (4)–(6) for HIV_+ people, setting $X_1 = S_1 + E_1 + I_1$ and $X_2 = S_2 + E_2 + I_2$, and noticing that the prevalence of HIV is $H = X_2/(X_1+X_2)$, we obtain the system

$$\frac{dX_1}{dt} = B - \mu_1 X_1 - f(H)\, H\, X_1 + (\mu_1 - m_1)\, I_1 , \tag{24}$$

$$\frac{dX_2}{dt} = -\mu_2 X_2 + f(H)\, H\, X_1 + (\mu_2 - m_2)\, I_2 . \tag{25}$$

To get a first estimation of d, λ and t_0, we neglect the terms involving I_1 and I_2 (active TB cases form a very small proportion of the population). The resulting system involves only X_1 and X_2, and it is formally the same as system (18) for HIV without TB. Taking

$X_1(t_0) = B/\mu_1$ and $X_2(t_0) = 1$, a good fit to HIV prevalence data from Table 1 is obtained with the parameters $d = 0.7$/year, $\lambda = 5.9$, and the year $t_0 = 1984$ for the beginning of the HIV epidemic. Three parameters are necessary and usually sufficient to fit any set of increasing numbers resembling the logistic curve, as is the case here. Recall that d, λ and t_0 cannot be taken from studies of other areas.

The parameter p_2 for fast progression to TB among HIV$_+$ people. In 1989, Di Perri et al. [19] studied an outbreak of TB among HIV$_+$ people: after the index case, eight people developed TB rapidly and six had a newly positive tuberculin skin test, suggesting that $8/14 \simeq 57\%$ of newly infected HIV$_+$ people develop primary TB disease. In 1992, Daley et al. [16] studied a similar outbreak and found a proportion equal to $11/15 \simeq 73\%$. But it is possible that only large outbreaks are studied, and that outbreaks with less cases of primary TB disease either are not noticed or are not a good subject for publication. A similar bias would occur if we based our estimate for the probability of fast progression to TB among HIV$_-$ people on reports of TB outbreaks such as the one investigated in [33], during which 14 out of 41 newly infected people (34%) developed primary disease. As a result, we prefer to let p_2 vary in order to fit the data concerning the TB notification rate from Table 4. For this purpose, we simulated system (1)–(6) starting from the initial condition

$$S_1(t_0) = S_1^*, \quad E_1(t_0) = E_1^*, \quad I_1(t_0) = I_1^*, \quad S_2(t_0) = 1, \quad E_2(t_0) = 0, \quad I_2(t_0) = 0.$$

Notice at this point that all the parameters in Table 1 have already been fixed except p_2. A relatively good fit was obtained with $p_2 = 30\%$ (plain line in Fig. 2a), i.e., nearly 3 times the value p_1 for HIV$_-$ people. Notice that this value for p_2 is still lower than the ones obtained by studying TB outbreaks among HIV$_+$ people [16,19]. Given the mortality μ_2 previously chosen for HIV$_+$ people, the estimates for a_2 and p_2 correspond to a probability $a_2/(a_2+\mu_2) \simeq 44\%$ of progressing slowly from latent to active TB and to a probability $p_2 + a_2/(a_2+\mu_2) \simeq 74\%$ of developing active TB after infection by MTB. As a comparison, the percentage of HIV$_+$ people that progress rapidly (either immediately or within 1 year) to active TB after infection by MTB was assumed to be 20% in [29] (no reference), 42% in [59] (no reference), 67% in [10] (citing [16]), and 100% in [70]. In models with a separate compartment for AIDS such as [20], the percentage was assumed to be 7% for early stage HIV (the same as for HIV$_-$ people) and 56% at the AIDS stage (citing [16,19]).

All the parameter values have now been fixed and are summarized in Table 6.

The percentage of HIV$_+$ TB notifications. The dashed line in Fig. 2a shows the contribution of HIV$_+$ people to the TB notification rate, as given by the simulation of the full model (1)–(6) with the parameters from Table 6. The curve passes close to the only data point we have (66% HIV$_+$ among TB notifications in 2005 [75]). This suggests that our parameter estimates are not unreasonable.

Checking the hypothesis used to estimate the HIV parameters d, λ and t_0. One can check if neglecting the terms involving I_1 and I_2 in (24)–(25) was reasonable. Figure 2b shows indeed that the simulation of the full model (1)–(6) with the parameters from

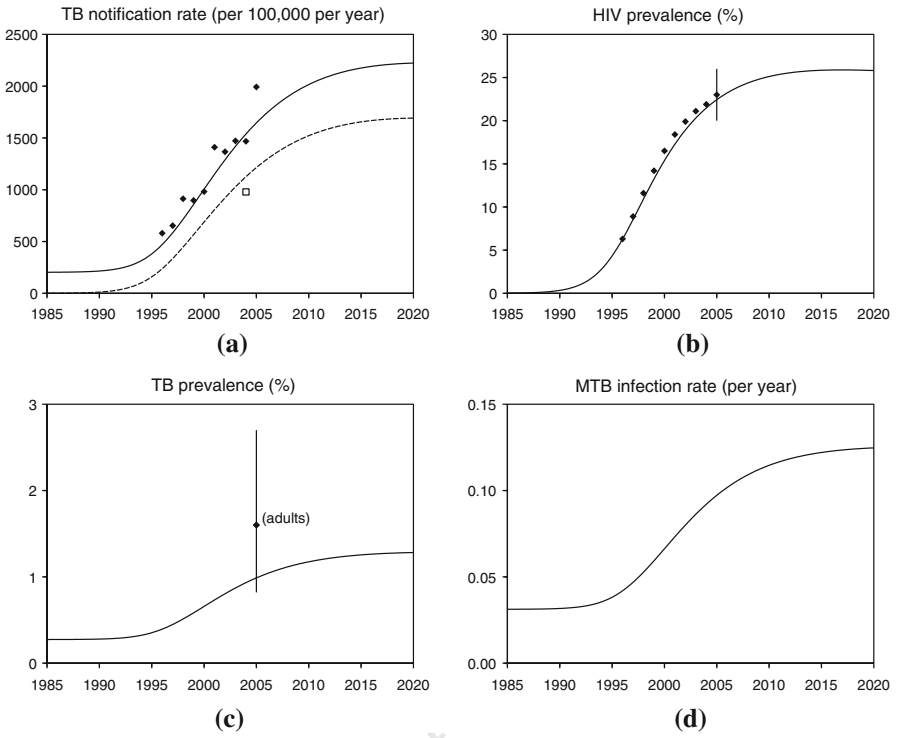

Fig. 2 **a** Data and simulation curve for the TB notification rate. The *dashed curve* shows the contribution of HIV_+ people (only one data point). **b** Data and simulation curve for HIV prevalence. **c** Simulation curve for the prevalence of active TB. The data point with 95% binomial CI corresponds to the prevalence of undiagnosed TB among adults, which is higher than for the whole population. **d** MTB infection rate

Table 6 Numerical values for the parameters of the model

	HIV_-			HIV_+		
Mortality	μ_1	0.02/year	[10]	μ_2	0.1/year	[10]
TB mortality	m_1	0.25/year	[13]	m_2	1.6/year	[13]
MTB infections	k_1	11.4/year	Fit	k_2	$k_1 \times 2/3$	[10]
Fast route	p_1	11%	[68]	p_2	30%	Fit
Slow route	a_1	0.0003/year	[68]	a_2	0.08/year	[3,61,62]
Reinfection	q_1	0.7 p_1	[68]	q_2	0.75 p_2	[10]
Recovery	β_1	0.25/year	[13]	β_2	0.4/year	[13]
Detection	γ_1	0.74/year	[12,75]	γ_2	3.0/year	[12,75]
Treatment	ε_1	80%	[75]	ε_2	80%	[75]
Births	B	200/year	[35]			
Contact rate	d	0.7/year	Fit			
Prevention	λ	5.9	Fit			
Initial year	t_0	1984	Fit			

Table 6 still gives a reasonably good fit to the HIV data. Notice that the data point with a 95% binomial confidence interval in Fig. 2b corresponds to the 23% HIV prevalence (174/762) in the sample population taken in the year 2005 [75].

Other curves. Figure 2c shows the prevalence of undiagnosed TB computed by simulating the full model (1)–(6) with the parameters from Table 6. The data point with a 95% binomial confidence interval corresponds to the prevalence of undiagnosed TB among adults (12/762), which should be higher than for the whole population. Hence, Fig. 2c also suggests that our parameter estimates are not unreasonable. Finally, we also show the MTB infection rate (Fig. 2d), for which data has been collected recently but has not yet been published. Recall, however, that our choice for the TB detection rates γ_1 and γ_2 was influenced by the knowledge that MTB infection rate had not risen as steeply as the TB notification rate.

7 Sensitivity of steady states with respect to changes in parameter values

All the parameter having been fixed or estimated (Table 6), we look at the numerical results following from the mathematical formulas of Sect. 4 for the steady states. First, the disease-free steady state with no HIV and no TB is $S_1^0 = 10,000$. We also obtain

$$R_0^{\mathrm{TB}} \simeq 1.3\,, \quad R_0^{\mathrm{HIV}} \simeq 7.0\,, \quad r_0^{\mathrm{TB}} \simeq 1.7\,, \quad r_0^{\mathrm{HIV}} \simeq 5.8.$$

The estimate $R_0^{\mathrm{TB}} \simeq 1.3$ is close to the range 0.6–1.2 mentioned in the review [50]. Using national HIV prevalence data from antenatal clinics, Williams et al. [71,73] found a similar result for R_0^{HIV}, namely 6.4 ± 1.6. Notice also that $r_0^{\mathrm{TB}} > R_0^{\mathrm{TB}}$: an "average" person newly infected with MTB will produce more secondary cases if introduced in a TB-free population where HIV is endemic than if introduced in a completely disease-free population. This is mainly because this "average" person is likely to be HIV_+, so its probability of progressing to active TB and of infecting other people is high (this depends on the numerical values of several parameters, including a_2, but not on the structure of the model). Finally, r_0^{HIV} is less than R_0^{HIV} as explained in Sect. 4.3. In some sense, TB slows down the HIV epidemic.

In the following subsections, we study the sensitivity of the different steady states with respect to the most important parameters of the model, namely those that enter in the nonlinear terms of system (1)–(6): the TB transmission rates k_1 and k_2, the reinfection parameters q_1 and q_2, and the parameters d and λ for HIV.

7.1 A global look at steady states in the (k_1, d) parameter space

Figure 3 shows a bifurcation diagram of the steady states in the (k_1, d) parameter space using the numerical values from Table 6 except of course for k_1 and d and assuming that the ratio k_2/k_1 is fixed. The black dot near the 2,000 per 100,000 per year level curve for the TB notification rate corresponds to the values of k_1 and d in Table 6. The boundaries between the four domains of the bifurcation diagram ("disease-free", "HIV", "TB", and "HIV + TB") are obtained by the solving the four

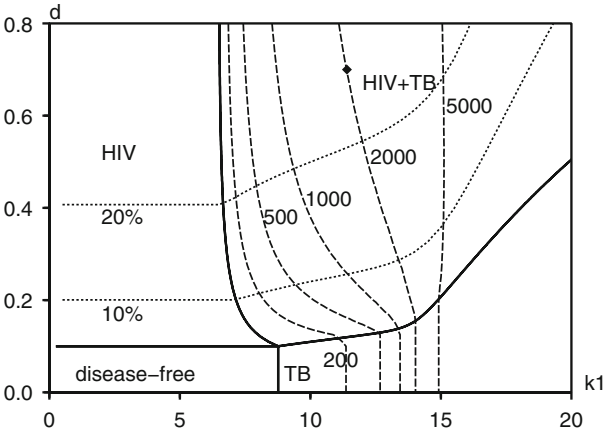

Fig. 3 Bifurcation diagram in the (k_1, d) phase plane and level curves of the steady state TB notification rate (*dashed lines*, 500 stands for 500 per 100,000 per year) and of the steady state prevalence of HIV (*dotted lines*)

equations $R_0^{\mathrm{HIV}} = 1$, $r_0^{\mathrm{HIV}} = 1$, $R_0^{\mathrm{TB}} = 1$ and $r_0^{\mathrm{TB}} = 1$ with respect to k_1 and d. Since R_0^{HIV} does not depend on k_1 and R_0^{TB} does not depend on d, the line $R_0^{\mathrm{HIV}} = 1$ is horizontal and the line $R_0^{\mathrm{TB}} = 1$ is vertical. The line $r_0^{\mathrm{HIV}} = 1$ separates "TB" from "HIV+TB". The line $r_0^{\mathrm{TB}} = 1$ separates "HIV" from "HIV+TB".

Notice in Fig. 3 how the level curves for the TB notification rate are distorted as they cross the line $r_0^{\mathrm{HIV}} = 1$ from the area labeled "TB" to the area labeled "HIV+TB". Notification rates near the "reinfection threshold" mentioned in Sect. 4.1 (for example the 1,000 and 2,000 level curves), which seemed totally unrealistic in the absence of HIV, occur now for smaller values of the transmission rate k_1 if HIV prevalence is high enough. With $k_1 = 11.4$ per year as in Table 6, the steady state TB notification rate increases from 200 to 2,000 per 100,000 per year as HIV prevalence increases from 0 to about 25%.

7.2 The steady state with TB but no HIV

The steady state with TB but no HIV is shown in the left part of Table 7. This is the steady state used as the initial condition in the simulations for the complete model with both HIV and TB. Notice that "Styblo's ratio" (for both smear-positive and smear-negative cases) is about 100, the value commonly admitted for HIV_- populations. Figure 4 shows how the TB steady state changes if we let k_1 and q_1 vary. The level curves of the steady state TB notification rate are also drawn. The black dot on the 200 per 100,000 per year level curve corresponds to the numerical values of k_1 and q_1 in Table 6. A similar "hand-drawn" picture without the level curves appears in [24, Fig. 3]. Some level curves cross each other in the zone of Fig. 4 with two positive solutions. They are the projections on the plane of level curves on a three-dimensional surface with a fold.

Table 7 Characteristics of the endemic steady state with TB only (Sect. 4.1) and with TB and HIV (Sect. 4.3)

	TB only	TB and HIV
Total population	9,695	4,161
Susceptible (HIV−) S_1	3,904	1,112
Latent TB (HIV−) E_1	5,764	2,029
Active TB (HIV−) I_1	27	30
Susceptible (HIV+) S_2	0	208
Latent TB (HIV+) E_2	0	762
Active TB (HIV+) I_2	0	20
HIV prevalence	0	24%
TB notification rate/100,000 per year	203	2,005
HIV_+ TB notifications	0	74%
MTB prevalence	60%	68%
TB prevalence	0.27%	1.2%
MTB infection rate/year	3.1%	12%
TB incidence rate/100,000 per year	299	2,945
"Styblo's ratio"	96	222
Reactivation (among TB cases)	6%	50%
Reinfection (among TB cases)	48%	32%
Primary progression (among TB cases)	46%	18%

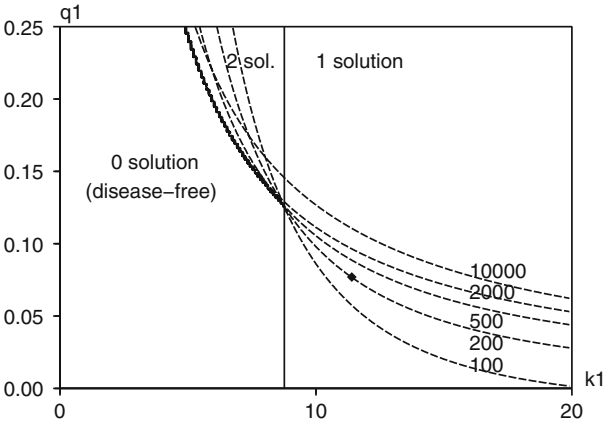

Fig. 4 TB only. Number of positive steady solutions of (7)–(9) in the parameter space (k_1, q_1). There is only one such solution to the *right of the vertical line* and either 0 or 2 to the left. The level curves of the steady state TB notification rate (per 100,000 per year) are also shown (*dashed lines*). Some level curves cross each other in the area with two solutions

Numerically, the threshold above which two positive steady states can exist is $q_1^* \simeq 12.5\%$, while we have chosen $q_1 = 7.7\%$. The other threshold separating the area where there are either 0 or 2 positive solutions from the area where there is 1 solution is $k_1^* \simeq 8.8$ per year, while our estimate is $k_1 = 11.4$ per year. The level curves in Fig. 4 show that the steady state TB notification rate is sensitive to variations

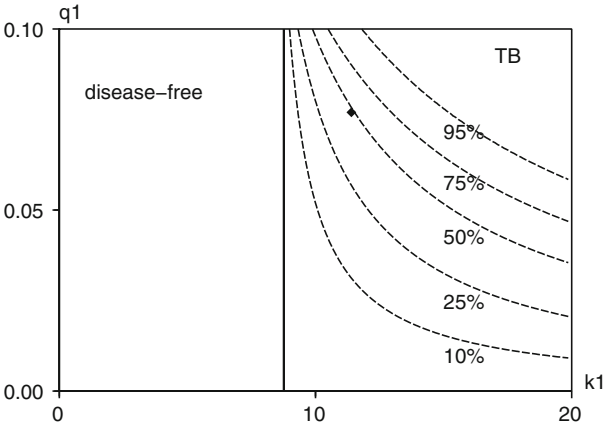

Fig. 5 TB only. Level curves of the percentage of new TB cases due to reinfection in the parameter space (k_1, q_1)

in q_1. This means that our estimation in Sect. 6 of the parameter k_1 (q_1 being fixed) should be considered with caution.

As noticed in [26,64] for a slightly different model, the dependence of the TB notification rate with respect to q_1 is even greater above a certain "reinfection threshold" (see the remarks at the end of the appendix and [7,27] for a dispute over this terminology). Notice for example how close the 2,000- and 10,000-level curves in Fig. 4 are. However, notification rates close to 2,000 per 100,000 per year as in the township under study here (with HIV) are already among the highest ever reported in a community. So it seems unlikely that TB parameter values for a community without HIV can be above the "reinfection threshold" as suggested in [26,64].

The percentage of new TB cases due to reinfection is shown as a function of k_1 and q_1 in Fig. 5. Notice that the vertical scale is not the same as in Fig. 4. A black dot indicates the numerical values for k_1 and q_1 from Table 6 that correspond to 45% of reinfection among new TB cases.

7.3 The steady state with HIV but no TB

In our model, the steady state with HIV but no TB is given by $\widehat{S}_1 \simeq 3{,}450$, $\widehat{S}_2 \simeq 1{,}310$, and $\widehat{H} \simeq 28\%$. The total equilibrium population with HIV is less than half of the disease-free steady state S_1^0, because we consider cohorts of B births per year and not the real total population with its inflows and outflows. The sensitivity of the steady state prevalence of HIV with respect to variations in λ and d are shown in Fig. 6. The black dot in the top right corner corresponds to the numerical values for λ and d from Table 6.

7.4 The steady state with both HIV and TB

The endemic steady state with both HIV and TB can be computed numerically. Its characteristics are shown in the right part of Table 7, and are those that would have

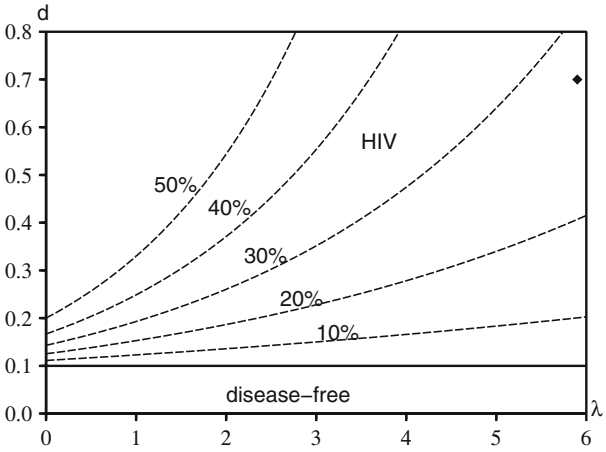

Fig. 6 HIV only. Bifurcation diagram in (λ, d) parameter space and level curves of the steady state HIV prevalence

been obtained if the simulations in Fig. 2 had been continued until reaching a steady state. Compared to the endemic steady state with TB only (left part of Table 7), the TB notification rate and the TB incidence have been multiplied by 10, the TB prevalence and the MTB infection rate by 4. The prevalence of MTB has only slightly increased. Reactivation has become the most important way of progression to active TB. The sensitivity of the steady state with both HIV and TB with respect to variations in k_1 or d was already shown in Fig. 3.

The question of whether the HIV-associated TB epidemic leads to an increased risk of MTB infection in the population (and in particular among HIV$_-$ people) has been a subject of discussion in the medical literature [22,38,74]. Egwaga et al. [22] found that the risk of infection had decreased between 1983 and 2003 in Tanzania among children aged 6–14 years despite the increase of HIV-associated TB incidence in the population. Similarly, Corbett et al. [11] did not find any increase in TB incidence among HIV$_-$ South African gold miners. On the contrary, Lawn and Wood [38] noticed that in the South African township under study here, the TB notification rate among HIV$_-$ adolescents had dramatically increased in recent years, so the risk of infection must have also increased. This is also what happens in our model: the MTB infection rate is multiplied by 4 as HIV prevalence increases from 0 to a steady state at 24%.

8 Control measures

8.1 Increasing condom use

Notice from (20)–(21) that r_0^{HIV} is proportional to $f(0) = d$ (the maximum transmission rate of HIV) and that r_0^{TB} is proportional to k_1 (the maximum transmission rate of TB), the ratio k_2/k_1 being fixed. So if d is divided by at least r_0^{HIV} (the other parameters being kept constant), the new r_0^{HIV} will be less than 1 and HIV will disappear

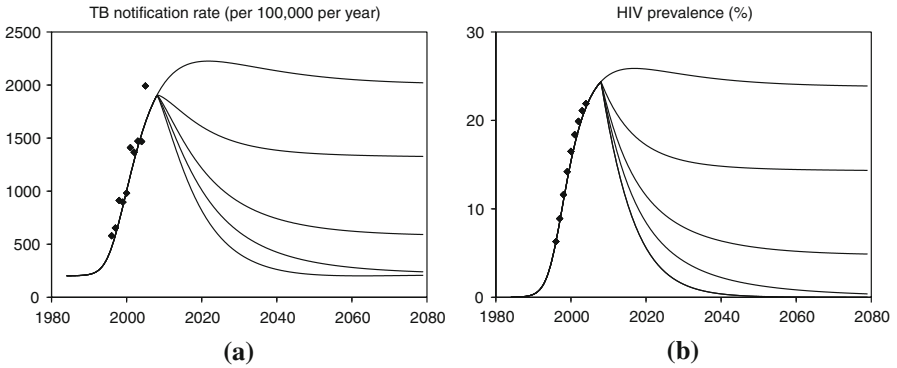

Fig. 7 Assuming that a sudden increase in condom use occurs in the year 2008 (the maximum transmission rate d becomes d'). The different curves correspond from *top* to *bottom* to $d' = d$, $d' = d/2$, $d' = d/4$, $d' = d/8$ and $d' = 0$. **a** TB notification rate. **b** Prevalence of HIV

in the long run. Similarly, if k_1 is divided by at least r_0^{TB}, the new r_0^{TB} will be less than 1 and TB will disappear in the long run. In Fig. 3, starting from the black dot representing the real situation, one can check that if k_1 is divided by $r_0^{\mathrm{TB}} \simeq 1.7$, we move from the area labeled "HIV+TB" to the area with HIV only. If d is divided by $r_0^{\mathrm{HIV}} \simeq 5.8$, we move from the area "HIV+TB" to the area with TB only. To decrease the parameter k_1, living conditions should be changed. The parameter d decreases if more condoms are used.

Figure 7 shows the impact of a sudden decrease of the HIV transmission rate d, from an initial value d to a new value d', on the prevalence of HIV (Fig. 7b) and also indirectly on the TB notification rate (Fig. 7a). The impact is obviously a monotonic function of d', as one would expect. We can check on these simulations that HIV disappears in the long run only if $d' < d/r_0^{\mathrm{HIV}} \simeq d/5.8$ (that is in the two simulations $d' = d/8$ and $d' = 0$ but not when $d' = d$, $d' = d/2$ or $d' = d/4$). If so, the TB notification rate returns finally to its level of the beginning of the 1980s, before HIV was introduced. The asymptotic TB notification rate and prevalence of HIV can also be read directly by looking at the level curves in Fig. 3, but the speed at which these steady states are reached can only be seen in Fig. 7.

In the absence of intervention (Fig. 7, $d' = d$), notice in the simulation that the peak for the prevalence of HIV occurs at about the same time as the peak for the TB notification rate. This does not seem incompatible with the data from Kenya [15, Fig. 1], which suggested a delay of several years between the rise of HIV and the rise of TB. One reason for such a delay may be that active TB tends to appear with a higher frequency in late stages of HIV infection. Notice, however, that the data from the South African township does not show any clear delay. Our model with just two compartments for HIV (HIV_- and HIV_+) could fit reasonably well the data for both TB and HIV although it does not include any delay. The background environments in the Kenyan study and in the South African township are probably quite different since for similar levels of HIV prevalence, the TB notification rate in Kenya is only one third of what it is in the South African township.

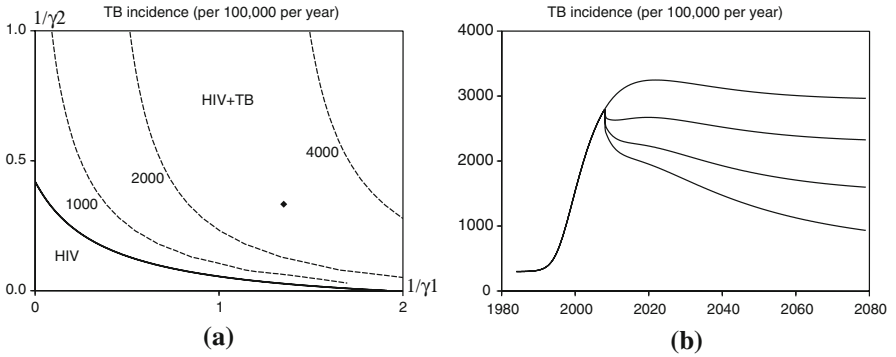

Fig. 8 Increasing the TB detection rate: **a** bifurcation diagram in the phase plane $(1/\gamma_1, 1/\gamma_2)$ and level curves of the TB incidence rate. **b** TB incidence rate as a function of time, assuming that a sudden increase in the TB detection rate for HIV_+ people occurs in the year 2008. The parameter γ_2 is replaced from top to bottom by γ_2, $2\gamma_2$, $4\gamma_2$ or $8\gamma_2$

Finally, one should mention that large scale prevention campaigns promoting condom use on television started at the end of the year 2006 in South Africa. In principle, one might be able to get data concerning the number of condoms purchased by the population of the township and check if behaviors have changed.

8.2 Increasing TB detection

Now we consider the possibility of increasing the TB detection rates γ_1 and γ_2 and increasing the probabilities ε_1 and ε_2 of successful treatment. For the township, this could be achieved by actively searching for TB cases instead of waiting for them to come to the TB clinic. Notice that the four parameters above enter the system of differential equations (1)–(6) only through the combination $b_1 = \beta_1 + \gamma_1 \varepsilon_1$ and $b_2 = \beta_2 + \gamma_2 \varepsilon_2$. However, we have to be a little careful because γ_1 and γ_2 enter in the expression of the TB notification rate (through $\gamma_1 I_1 + \gamma_2 I_2$). If γ_1 or γ_2 increase, the steady state TB notification rate may increase and will start decreasing only if γ_1 or γ_2 are high enough. It is therefore not suitable to use the TB notification rate as a measure of the severity of the situation when the detection rate changes. Instead, we will use the TB incidence rate.

Figure 8a shows the bifurcation diagram and the level curves of the steady state TB incidence rate in the parameter space $(1/\gamma_1, 1/\gamma_2)$, using the numerical values from Table 6 for the other parameters. Since γ_1 and γ_2 do not enter in the formula for R_0^{HIV}, the HIV-endemic steady state is always there. The question is: when can it be invaded by TB? This is given by the equation $r_0^{\text{TB}} = 1$, an implicit equation for γ_1 and γ_2 shown by the thick black line separating "HIV" from "HIV+TB" in the bottom left corner of Fig. 8a. The values for γ_1 and γ_2 in Table 6 correspond to the black dot shown in the figure.

Figure 8b shows the impact of a sudden increase in the TB detection rate γ_2 for HIV_+ people. This has almost no impact on the curve for the prevalence of HIV so we

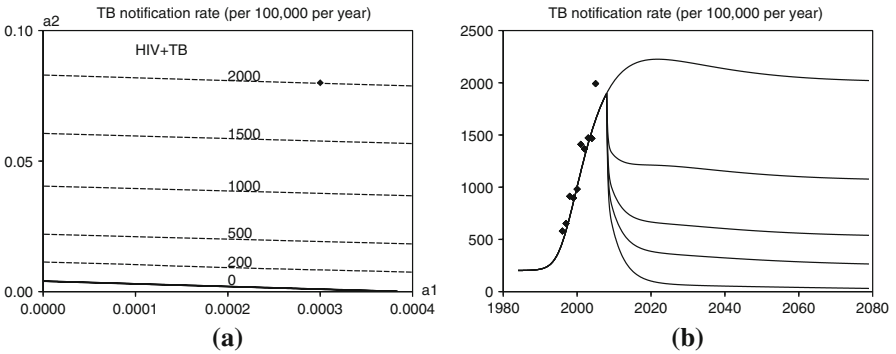

Fig. 9 Isoniazid preventive therapy for HIV_+ people (decreasing a_2): **a** bifurcation diagram in the phase plane (a_1, a_2) and level curves of the steady state TB notification rate. **b** TB notification rate as a function of time. Assumption: starting in 2008, a_2 is replaced from *top* to *bottom* by a_2, $a_2/2$, $a_2/4$, $a_2/8$ or 0

do not show it. Of course, the TB incidence decreases monotonically as the detection rate increases.

8.3 Isoniazid preventive therapy

This control measure reduces the parameter a_1 if used for HIV_- people and the parameter a_2 if used for HIV_+ people. These parameters do not enter in the formula for R_0^{HIV}, so HIV is always present and the question is whether TB can be stopped in the presence of HIV: the threshold is given by $r_0^{TB} = 1$ (the corresponding curve appears in the bottom of Fig. 9a as the level set 0). The level curves of the TB notification rate in the diagram (a_1, a_2) are almost horizontal (Fig. 9a). So preventive therapy used for HIV_+ people (reducing a_2) has a much greater impact on the TB notification rate than if used for HIV_- people (reducing a_1). The values for a_1 and a_2 in Table 6 correspond to the black dot in Fig. 9a close to the 2,000 per 100,000 per year level curve.

Figure 9b shows the impact of a sudden decrease of the progression rate a_2 for HIV_+ people due to isoniazid preventive therapy. Since this has almost no impact on the curve for the prevalence of HIV, we do not show it. The steady state TB notification rate decreases monotonically as a_2 decreases.

8.4 ART

We consider now the possible impact of antiretroviral treatment (ART), more precisely, of highly active antiretroviral treatment (HAART). ART reduces viral load and therefore also the transmission parameter d for HIV. But ART also increases the life expectancy of HIV_+ people by decreasing μ_2 and m_2 (of course not below the natural mortality μ_1), a fact which increases the number of people living with HIV and enhances further transmission of HIV. These two effects are antagonistic, so the impact on HIV at the population level is not obvious and depends very much on how much each of the three parameters involved changes with ART. Besides, ART reduces the

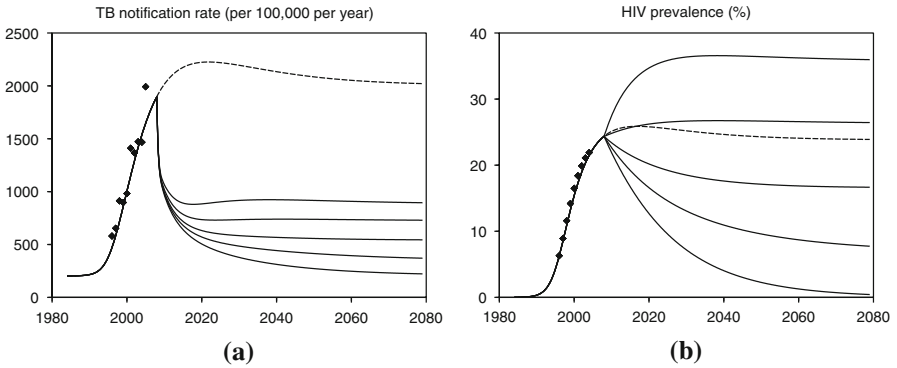

Fig. 10 ART. *a* TB notification rate as a function of time. *b* HIV prevalence as a function of time. Assumption: 100% of HIV_+ people are put on ART starting in 2008. The parameter μ_2 is replaced by $\mu_2/2$, the parameter m_2 by $m_2/2$, the parameter a_2 by $a_2/5$, while the parameter d is replaced either by d, $d/2$, $d/4$, $d/8$, or 0 (from *top* to *bottom*). The *dashed line* shows the case without intervention

average rate a_2 at which coinfected people develop active TB, though not to the same level a_1 as HIV_- MTB-infected people [3,34,36,39], and even if "immune reconstitution disease" may on the contrary increase a_2 during the first few months of ART treatment [41]. Again, the effect of ART on TB is not clear because HIV_+ people under ART live longer. Quantitatively, ART was shown in studies in South Africa [3,36] and Brazil [45] to reduce a_2 by 80% , i.e., to divide a_2 by 5. With $a_2 = 0.08$ per year without ART, this gives $a_2 = 0.016$ per year under ART. This is still 50 times higher than the parameter $a_1 = 0.0003$ per year for HIV_- people. Another report [37] mentioned a risk 5 to 10 times higher after 3 years of ART compared to HIV_- people. We assume furthermore that:

- μ_2 is divided by 2 under ART, giving $\mu_2 = 0.05$ per year instead of 0.1 per year, still higher than the natural mortality $\mu_1 = 0.02$ per year; the new life expectancy for HIV_+ people under ART is 20 years;
- m_2 is divided by 2 under ART (the new m_2 is 0.8 per year, compared to $m_1 = 0.25$ per year).

We determined what would happen under various assumptions for the HIV transmission parameter d (Fig. 10), assuming that 100% of HIV_+ people are put immediately on ART starting in 2008, independently of their CD4 cell count (a variable which is not included in our model anyway). This hypothesis is of course quite optimistic and would require the entire adult population of the township to be tested for HIV. Notice also that in practice and in more realistic models, some factors may favour a delayed initiation of ART [40]. With our choice of parameter values, we find a decrease for the TB notification rate even in the extreme case where ART would have no influence on the parameter d (Fig. 10a, top plain curve), a case which would lead to an increase in HIV prevalence (Fig. 10b, top plain curve). The cases where $d' = d/2$ and $d' = d/4$ are probably more realistic, since we expect HIV transmission to decrease if everybody knows his/her HIV status. In such cases (and assuming that the other parameters values have been correctly chosen), HIV prevalence would decrease for

$d' = d/4$ but not for $d' = d/2$ (Fig. 10b, second and third plain curves from the top). So the future of HIV prevalence under ART is uncertain. But with a progression rate a_2 reduced by 80% and a life expectancy $1/\mu_2$ multiplied by 2, it seems that ART would dramatically decrease the TB notification rate even though the new reactivation rate for HIV_+ people would still be several times higher than the one for HIV_- people.

ART has become increasingly available in the township since 2006. But it is still too early to understand what its impact on both the HIV and TB epidemics has really been.

9 Conclusion

This work is a first attempt to model the simultaneous HIV and TB epidemics in a township near Cape Town, South Africa, for which a considerable amount of data is available. The main difficulty is due to the large number of parameters in the model, which makes estimations and mathematical analysis a little difficult. Keeping this number as small as possible, we have been able to provide a fairly complete picture of the model with HIV or TB only.

Backward bifurcation for our model with TB only was shown to be impossible under realistic parameter values because MTB infection provides a certain degree of protection against a fast progression to active TB after reinfection ($q_1 \leq p_1$). To our knowledge, no TB model has ever been shown to exhibit backward bifurcation under realistic parameter values despite all the emphasis put on this possibility in the more mathematically oriented articles on TB [24,48,63]. On this point, we agree with Lipsitch and Murray [42] and with Singer and Kirschner [64].

For the full model (1)–(6) with both HIV and TB, we analyzed the linear stability of the endemic steady states with either TB or HIV. We conjectured that there was still no backward bifurcation for (1)–(6) when $q_1 \leq p_1$. Verifying this point can be considered as an open mathematical problem. We used numerical methods to draw bifurcation diagrams with level curves for HIV prevalence and TB notification rate. The most interesting diagram is Fig. 3. It shows how for a fixed value of the TB transmission rate k_1, the steady state TB notification rate can increase from 200 to 2,000 per 100,000 per year as HIV prevalence increases from 0 to around 25%.

Gomes et al. [26–28] have emphasized the role of a "reinfection threshold" in TB models without HIV. In [26, Fig. 3] or [28, Fig. 2], the "reinfection threshold" occurred when approximately 1% of the population had active TB. In the South African township, $12/762 \simeq 1.6\%$ of a sample population was found to have undiagnosed active TB in 2005. This could suggest that there are indeed populations above the "reinfection threshold". However, one can wonder if populations with endemic TB but with low HIV prevalence can really reach 1% prevalence of active TB. In other words, one can wonder if such populations are not systematically below the "reinfection threshold", and if the "reinfection threshold" can still be used to explain problems in TB epidemiology such as the inefficiency of BCG vaccination [26]. Even in populations with high HIV prevalence, the "reinfection threshold" does not seem to play such an important role. Table 7 suggests that the percentage of reinfection with HIV and TB is less than with TB only.

Among the control measures studied, most have an obvious positive impact in controlling the HIV or TB epidemics: this is the case for condom use, increased TB detection and preventive treatment. The situation for ART is more complicated. However, although the future for the prevalence of HIV is uncertain, it seems that a generalized access to ART would lead to a significant decrease of the TB notification rate. Indeed, ART has been shown both in South Africa and in Brazil to reduce the progression rate from latent to active TB by about 80%, i.e., to divide it by a factor 5. If HIV_+ people under ART live approximately 2 times longer than the average 10-year survival time of HIV_+ people with no access to ART, then one could expect the TB incidence to be multiplied by $0.4 = 2/5$, i.e. to be reduced by 60%. This simple argument may be wrong if ART increases the prevalence of HIV and indirectly the incidence of TB. Our numerical results suggest that this is not so. Even in the worst scenario we considered where HIV prevalence increased as a result of ART (top plain curve in Fig. 10b), the TB notification rate decreased considerably (top plain curve in Fig. 10a).

It is difficult to guess if the observations drawn from this model with parameters adapted to this particular South African township are still valid for less crowded areas with high HIV prevalence. One could try to use the same model and adapt the parameters to data from such areas. Unfortunately, reliable data on both HIV and TB is still rare. For example, HIV prevalence in Zimbabwe has probably not been estimated as regularly as [32, Fig. 5] might suggest (J. Hargrove, personal communication).

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Appendix

Let us call i_1^+ and i_1^- the two (possibly complex) roots of Eq. (13), which for convenience we rewrite as

$$\left(i_1^*\right)^2 + c_1\, i_1^* + c_0 = 0\,. \tag{26}$$

We are only interested in positive roots, which are the ones with a biological meaning. The existence of positive roots depends in particular on the signs of c_1 and c_0. We need to distinguish several cases:

- The case $k_1 > k_1^*$. Since $c_0 < 0$, it follows that $i_1^+ \times i_1^- < 0$. This case occurs only if $i_1^+ > 0$ and $i_1^- < 0$. So there is only one positive solution of (13).
- The case where $0 < k_1 < k_1^*$ and $0 < k_1 < \widetilde{k}_1(q_1)$, the new parameter $\widetilde{k}_1(q_1)$ being defined by

$$\widetilde{k}_1(q_1) = \frac{a_1 + b_1 + (1-p_1)\,m_1 + p_1\mu_1}{q_1} + m_1\,. \tag{27}$$

In this case we have $c_1 > 0$, so $i_1^+ + i_1^- < 0$. But $i_1^+ \times i_1^- > 0$, so this case occurs only if i_1^+ and i_1^- are both negative or if i_1^+ and i_1^- are complex conjugates with a negative real part. So there is no positive solution of (13). Notice that $\widetilde{k}_1(q_1) < k_1^*$ only when $q_1 > q_1^*$, the definition (17) of q_1^* having been precisely chosen for this purpose.

- The case where $0 < k_1 < k_1^*$ and $k_1 > \widetilde{k}_1(q_1)$ (which implies that $q_1 > q_1^*$). Let Δ be the discriminant of (13). Let us emphasize its dependence on k_1 by writing $\Delta(k_1)$ while we keep q_1 fixed. From (13), we see that $\Delta(k_1)$ is a quadratic polynomial with respect to $1/k_1$, so the equation $\Delta(k_1) = 0$ has at most two roots in the half-line $k_1 > 0$. Since $\Delta(k_1) = c_1^2 - 4\,c_0$, since $c_1 = 0$ when $k_1 = \widetilde{k}_1(q_1)$ and $c_0 = 0$ when $k_1 = k_1^*$, it follows that $\Delta(\widetilde{k}_1(q_1)) < 0$ and $\Delta(k_1^*) > 0$. So the equation $\Delta(k_1) = 0$ has at least one root in the interval $(\widetilde{k}_1(q_1), k_1^*)$, and it cannot have two roots since the function $k_1 \mapsto \Delta(k_1)$ has to change sign an odd number of times in this interval. Call $\widehat{k}_1(q_1)$ the unique root. Then $\Delta(k_1) < 0$ for $\widetilde{k}_1(q_1) < k_1 < \widehat{k}_1(q_1)$: in this case, Eq. (13) has no real solution. For $\widehat{k}_1(q_1) < k_1 < k_1^*$, we have $\Delta(k_1) > 0$, $c_0 = i_1^+ \times i_1^- > 0$, and $c_1 = -(i_1^+ + i_1^-) < 0$: in this case, Eq. (13) has two positive solutions.

We still have to check that if (13) has a positive root i_1^*, then all the components of the triplet (S_1^*, E_1^*, I_1^*) given by (14)–(15) are positive. For this purpose, it is enough to show that $s_1^* > 0$ and $e_1^* > 0$. But adding (8) and (9), we find that $s_1^* = (\mu_1 e_1^* + m_1 i_1^*)/(k_1 i_1^*)$. So it is enough to show just that $e_1^* > 0$, i.e., $i_1^* < 1 - m_1/k_1$. Notice first from (27) that if (13) has a positive root i_1^*, then $k_1 > m_1$ necessarily holds. Let us call $\chi(i_1)$ the quadratic polynomial on the left of (13), so that $\chi(i_1^*) = 0$. Simple computations show that

$$\chi(1 - m_1/k_1) = \frac{[b_1 + (1-p_1)\,m_1]\,(k_1 - m_1 + \mu_1)}{q_1 k_1^2} > 0\,,$$

$$\chi'(1 - m_1/k_1) = 1 - \frac{m_1}{k_1} + \frac{a_1 + b_1 + (1-p_1)m_1 + p_1\mu_1}{q_1 k_1} > 0\,,$$

which imply that $i_1^* < 1 - m_1/k_1$. Q.E.D.

Finally, let us add a short comment on the notion of "reinfection threshold" [7,26,27] for our model in the case $k_1 > k_1^*$ (which is equivalent to $R_0^{\mathrm{TB}} > 1$ and also to $c_0 < 0$). The unique positive solution of (26) is

$$i_1^* = \left[-c_1 + \sqrt{c_1^2 - 4\,c_0}\right]/2\,.$$

Consider the special case where $4|c_0|/c_1^2$ is small. This case turns out to be satisfied numerically in the whole area $k_1 > k_1^*$ of Fig. 4 except in a very narrow strip around the curve $c_1 = 0$, whose equation can be rewritten as

$$q_1 = Q_1(k_1) = \frac{a_1 + b_1 + (1-p_1)m_1 + p_1\mu_1}{k_1 - m_1}\,.$$

Then one can show that

$$i_1^* \simeq \begin{cases} -c_0/c_1 & \text{if } c_1 > 0\,, \\ -c_1 & \text{if } c_1 < 0\,. \end{cases}$$

The approximation for $c_1 > 0$ corresponds to neglecting the quadratic term in Eq. (26), while the approximation for $c_1 < 0$ corresponds to neglecting the constant term. Notice that because $4|c_0|/c_1^2$ was assumed to be small, the expression $-c_0/c_1$ is much smaller than $-c_1$. So the prevalence of TB is high when $q_1 > Q_1(k_1)$ and much smaller when $q_1 < Q_1(k_1)$. But as pointed out in [7,27] for a slightly different model, the "reinfection threshold" we have just obtained is not very well defined from a mathematical point of view (a similar situation happens e.g. when defining the width of "boundary layers" in physics).

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Molecular Epidemiology of *Mycobacterium tuberculosis* in a South African Community with High HIV Prevalence

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To explore the relationship between human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* genotypes, we performed IS*6110*-based restriction fragment–length polymorphism analysis on *M. tuberculosis* culture specimens from patients with smear-positive tuberculosis in a periurban community in South Africa from 2001 through 2005. Among 151 isolates, 95 strains were identified within 26 families, with 54% clustering. HIV status was associated with W-Beijing strains ($P = .009$) but not with clustering per se. The high frequency of clustering suggests ongoing transmission in both HIV-negative and HIV-positive individuals in this community. The strong association between W-Beijing and HIV infection may have important implications for tuberculosis control.

Tuberculosis remains a major cause of morbidity and mortality worldwide and in Africa human immunodeficiency virus (HIV) is fueling the epidemic [1]. South Africa, which bears 28% of the global burden of HIV-related tuberculosis, is undergoing rapid urbanization [2], with immigrants to the cities concentrating in poor, crowded periurban townships where both HIV prevalence and tuberculosis incidence rates are high [3]. We have previously described high HIV prevalence in adults (23%) [4] and rapidly escalating tuberculosis notification rates [3] in a periurban township in Cape Town, South Africa. Despite a well-implemented national tuberculosis control program (based on the World Health Organization's DOTS [directly observed therapy, short course] strategy [5]), the number of tuberculosis cases has escalated at the single community clinic that manages all of this township's resident patients with tuberculosis. This geographically well-defined community with ~15,000 people of low socioeconomic status living in overcrowded, largely informal dwellings is ideally suited for tuberculosis transmission studies.

The advent of such molecular epidemiological tools as IS*6110*-based restriction fragment–length polymorphism (RFLP) genotypic analysis of *Mycobacterium tuberculosis* has expanded our ability to investigate and understand tuberculosis [6]. Different *M. tuberculosis* strains have been associated with diverse levels of virulence, immunological responses, epidemic potential, and even drug resistance [6]. However, the relationship between specific host characteristics (such as HIV infection) and *M. tuberculosis* genotypes is poorly understood. To explore the association between HIV infection and circulating *M. tuberculosis* strains, we performed RFLP analysis of *M. tuberculosis* isolates cultured from individuals with smear-positive pulmonary tuberculosis in the above-mentioned study community from 2001 through 2005.

Methods. Sputum specimens from patients with acid-fast bacilli smear–positive tuberculosis residing in the study community were collected for genotype analysis from 2001 through 2005. Sputum specimens were obtained from patients in accordance with National Tuberculosis Control Program guidelines [7] and were labeled as study specimens at the clinical site for identification by laboratory personnel. The age, sex, details of clinical diagnosis, clinical outcome, and HIV status of each patient were collected from the clinic's tuberculosis register and patient folders. The study was approved by the ethics review boards of the University of Cape Town and of the University of Medicine and Dentistry of New Jersey (UMDNJ), and participants provided informed consent.

Sputum specimens were assessed for the presence of *M. tuberculosis* bacilli by means of fluorescent (auramine) microscopy. Isoniazid and rifampicin susceptibility testing was performed on samples from retreatment patients and from patients whose samples still tested positive for acid-fast bacilli after 2

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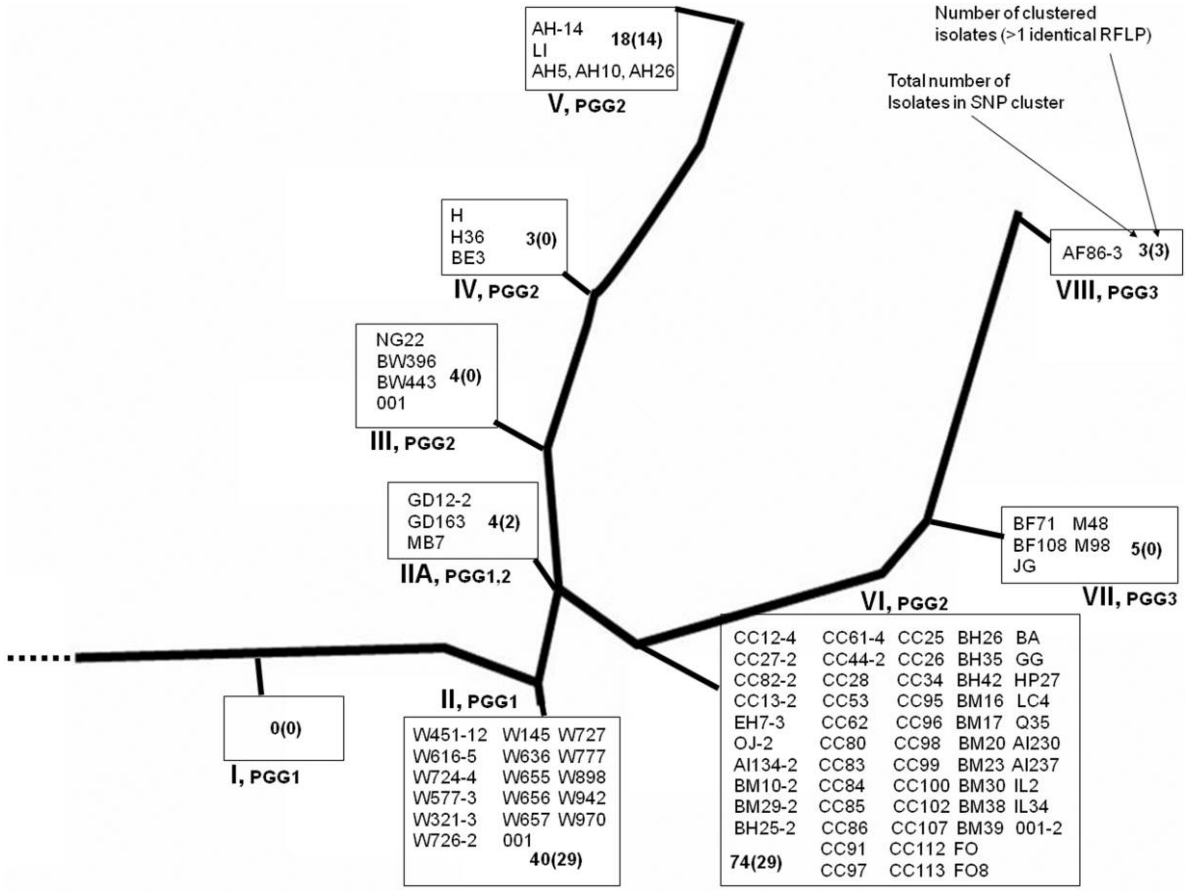

Figure 1. *Mycobacterium tuberculosis* strains from the study community in a phylogenetic framework. RFLP, restriction fragment–length polymorphism; SNP, single-nucleotide polymorphism.

months of treatment [7]. Susceptibility to rifampicin was tested at a concentration of 1.0 μg/mL, and susceptibility to isoniazid was tested at concentrations of 0.1 and 0.2 μg/mL on mycobacterial growth indicator tubes and on Middlebrook 7H11 agar, respectively. Sputum samples that tested positive for acid-fast bacilli were cultured on Lowenstein-Jensen slants at the Tuberculosis Laboratory at the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town. Isolates that tested positive for *M. tuberculosis* by culture were inoculated in duplicate into 7H9 liquid medium supplemented with oleic acid, albumin, dextrose, and catalase (OADC) and 15% glycerol, then stored at −70°C.

Frozen duplicate culture stock was shipped to the Public Health Research Institute (PHRI) Tuberculosis Center at UMDNJ. Culture stocks were subcultured on Lowenstein-Jensen slants, and DNA was extracted from each isolate. IS*6110*-based RFLP analysis was performed as described elsewhere [8]. RFLP patterns were analyzed using Bio Image pattern matching software (Bio Image). *M. tuberculosis* isolates that had DNA fingerprints with an identical hybridization banding pattern were considered to be the same strain and assigned a strain code following a nomenclature system that has been described elsewhere [9]. *M. tuberculosis* strains were assigned to 1 of 9 (I–VIII and II.A) discrete synonymous single-nucleotide polymorphism (SNP)–based phylogenetic lineages (synonymous SNP clusters) that were inferred on the basis of RFLP patterns and of previous analysis, reported elsewhere [6], of *M. tuberculosis* clinical isolates at PHRI. In addition, strains that exhibited similar IS*6110* hybridization profiles ($\geq$65% similarity), suggesting common recent ancestry, were collectively grouped into genotype families (eg, W-Beijing, CC, and BM) [6]. Strain clusters were defined as >1 occurrence of a specific strain during the study period. Strain patterns that were represented only once in the PHRI database and did not qualify for a family assignment were considered unique and given a default assignment (001).

Data were analyzed using Stata software (version 10.0; StataCorp). Bivariate analyses used the Student *t*, χ^2, and Fisher exact tests, as appropriate. A Wilcoxon rank sum test was used for comparison of median age between different groups. Patients' ages were categorized by decade (15–19, 20–29, 30–39, 40–49, 50–59, and $\geq$60 years of age) for analysis of the dis-

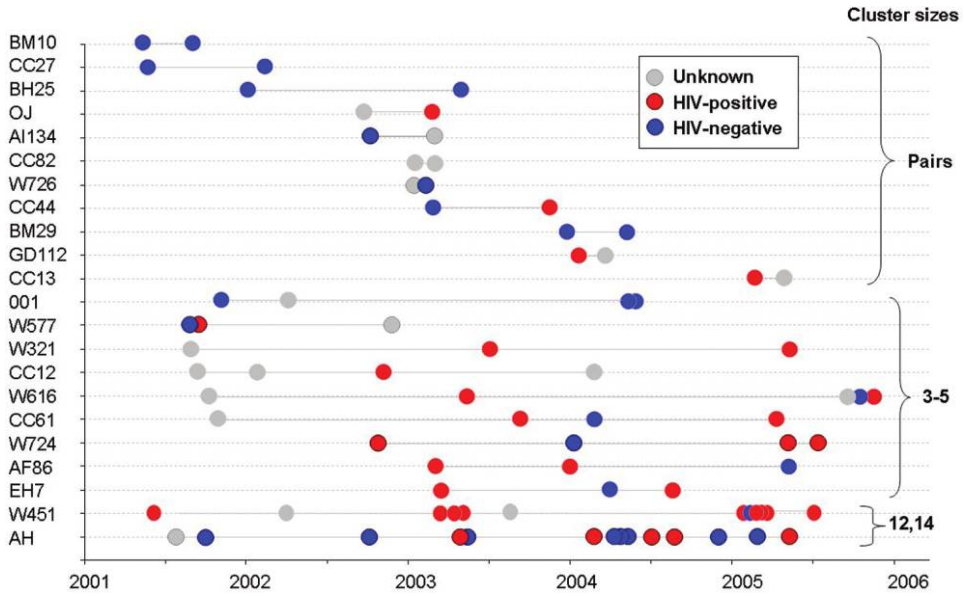

Figure 2. Distribution of the clusters, by cluster size, date of diagnosis, human immunodeficiency virus (HIV) status, and *Mycobacterium tuberculosis* strain family.

tribution of the 4 main *M. tuberculosis* families by age. A χ^2 test for trend was used to assess changes in strain distribution over time.

Multiple logistic regression models were developed to examine factors associated with the dominant strain families and with clustering of strains. The period between occurrences of cases within clusters was calculated on the basis of the date of tuberculosis diagnosis. The ArcMap (version 9.2; Esri) geographic information system was used to assess the spatial distribution of *M. tuberculosis* strains occurring in clusters during the course of the study.

Results. Over the 5-year study period, 467 patients in the study community were diagnosed with sputum smear–positive pulmonary tuberculosis. The study laboratory received sputum specimens from 282 patients (60% of smear-positive patients) over this period. Of the 282 specimens received from these patients, 53% ($n = 149$) were successfully cultured for RFLP analysis, 22% ($n = 61$) lost viability during shipping or storage, 19% ($n = 55$) failed to culture *M. tuberculosis*, and 6% ($n = 17$) were contaminated. Two patients had dual infections with 2 different strains, and therefore a total of 151 *M. tuberculosis* isolates were included in this analysis.

No statistically significant differences were found between patients with and those without RFLP data (including patients for whom we did not receive specimens) in terms of age ($P = .11$), sex ($P = .63$), tuberculosis category (ie, new or retreatment cases; $P = .22$), test results for multidrug-resistant (MDR) tuberculosis (resistant to at least isoniazid and rifampicin; $P = .15$) or HIV status (among those tested; $P = .58$). However, patients for whom we did not obtain RFLP data had a higher death rate than did those for whom we did obtain RFLP data ($P < .001$).

The 149 patients with RFLP results ranged in age from 14 to 67 years (median age, 36 years), and 62% ($n = 92$) were men. In total, 81% ($n = 121$) of the patients were tested for HIV, and 54% ($n = 65$) of those 121 patients tested were infected with HIV. Four of the 149 patients (3%) had confirmed MDR tuberculosis. There were no tuberculosis-related deaths in this cohort.

A total of 95 different *M. tuberculosis* strains were identified (including 4 unique isolates), which are presented in a phylogenetic framework in Figure 1. Eight of the 9 recognized synonymous SNP clusters [10] were present in the community. The synonymous SNP cluster VI comprised 49% ($n = 74$) of the patients. Genetic variability within this group was high, with 57 strains detected in 74 patients. The synonymous SNP cluster II was the second largest group, with 26% ($n = 39$) of strains.

Twenty-six different *M. tuberculosis* genotype families were identified; the 4 largest families were W-Beijing (accounting for 26% of all *M. tuberculosis* strains in the community), CC (25%), AH (11%), and BM (7%). Bivariate analysis revealed no statistically significant association between the 4 dominant families and sex ($P = .56$), age category ($P = .31$), tuberculosis category ($P = .73$), or outcomes of tuberculosis treatment ($P = .53$). There was no change in the distribution of the main strain families across the 5-year period of data collection ($P = .54$).

Multivariate analysis adjusting for age, sex, tuberculosis category, and outcome yielded no association between HIV status and CC strain (odds ratio [OR], 1.23 [95% confidence interval

{CI}, 0.48–3.17]), AH strain (OR, 0.68 [95% CI, 0.21–2.25]), or BM strain (OR, 0.26 [95% CI, 0.04–1.49]). However, patients with the W-Beijing strain were significantly more likely to be HIV positive (OR, 3.65 [95% CI, 1.37–9.71]).

Of the patients with MDR tuberculosis, 2 were infected with W-Beijing strains, 1 was infected with a CC strain, and 1 was infected with an H strain. There was no statistical association between MDR tuberculosis and strain ($P = .67$) or HIV status ($P = .87$).

In this study, 54% ($n = 81$) of isolates occurred in strain clusters ranging in size from 2 to 14 patients, with 27% of the clustered strains occurring in pairs. Figure 2 demonstrates the distribution of the clustered strains over time by HIV status. In multivariate analysis, there was no association between clustering and HIV status (OR, 1.20 [95% CI, 0.55–2.65]). Paired clusters were diagnosed on average 157 days apart (<6 months), compared with an average of 321 days (>10 months) between occurrences of cases in the larger clusters ($P = .013$). Smaller, temporally associated clusters were noted within the larger clusters of the W451 and AH strains. With the exception of 2 cases in the AH cluster, none of the clustered cases occurred on the same residential plot in the township studied.

Discussion. The key finding of this study is the association between W-Beijing, one of the largest identified *M. tuberculosis* strain families, and HIV infection. This association persisted after controlling for a number of clinical factors and could be due to either an increased pathogenicity or virulence of the strain or an increased susceptibility of HIV-infected patients to these strains. W-Beijing *M. tuberculosis* strains have shown marked virulence in animal models of infection [6] and it has been suggested that certain sublineages of W-Beijing may have increased transmissibility and/or pathogenicity [11]. However, to our knowledge this is the first population-based study to show an association between W-Beijing and HIV infection. Further study of the biology of the W-Beijing strains and of their interaction with the HIV-infected host may help to explain the increased susceptibility of HIV-infected patients to tuberculosis and may also indicate novel ways to either protect or more effectively treat HIV-infected patients coinfected with *M. tuberculosis*. Other studies have found W-Beijing strains to be associated with MDR tuberculosis [12]. Therefore, the association with HIV-infected patients may have serious implications for the spread of MDR tuberculosis. The increased mortality in those without RFLP analysis may reflect a lower sputum retrieval rate at the local hospital, where tuberculosis was initially diagnosed in sicker patients.

This study has demonstrated a broad diversity of different *M. tuberculosis* strains, consistent with findings of other studies in sub-Saharan Africa [13, 14]. The high degree of genotypic diversity within the CC strains (related to F11/LAM) [13] may indicate that they are endemic in this population. The W-Beijing family also shows a high degree of diversity (16 variants in 39 patients), although to a lesser degree than the CC family, suggesting that these strains may be emerging and diversifying in the community. However, chromosomal location of IS*6110* insertions may affect the movement of the insertion sequence elements, and therefore genetic diversity as determined by IS*6110* RFLP may be independent of strain endemicity.

Another finding was the high rate of strain clustering. Clustering is not necessarily synonymous with recent transmission; evidence of geographical linkage, temporal association, and social contacts may be needed to support the suggestion of transmission. IS*6110* fingerprinting is one of the most discriminatory typing techniques for isolates with >6 IS*6110* bands (eg, CC and W-Beijing), although it is less discriminatory for strains with fewer bands (eg, AH) [6], and may underestimate subclusters within the AH family. In this study approximately half of the strains were clustered, and there were close temporal associations, especially among the paired clusters. A proportion of disease may therefore be due to new infections. Because no association was found between HIV infection and clustering, new infections may be occurring in both HIV-negative and HIV-positive patients. Given the incomplete sampling of the study population, we probably underestimated the number of circulating strains, number of clusters, and sizes of clusters [15].

The spatial analysis demonstrated that temporally linked clusters did not occur on the same residential plots, suggestive of transmission outside of households. Temporally related clustering was also identified within larger clusters, such as of the W451 and AH strains. The strong temporal relationship of tuberculosis in HIV-positive patients within the W451 strain cluster (4 patients in 2003 and 6 patients in 2005) (Figure 2) may reflect nosocomial transmission at the single community clinic. Traditional epidemiological studies, including social interaction studies, are required to further delineate transmission.

In conclusion, we have shown that a wide diversity of strains exists in this periurban community in South Africa. Strain clustering, suggestive of ongoing transmission, was common in both HIV-positive and HIV-negative adults. The W-Beijing family was associated with HIV infection, a new finding that requires confirmation and explanation.

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Childhood tuberculosis infection and disease: A spatial and temporal transmission analysis in a South African township

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Background. Tuberculosis (TB) remains a leading cause of mortality and morbidity in South Africa. While adult TB results from both recent and past infection, childhood TB results from recent infection and reflects ongoing transmission despite current TB control strategies.

Setting. A South African community with high rates of TB and HIV disease.

Outcomes. A Geographic Information System was used to spatially and temporally define the relationships between TB exposure, infection and disease in children <15 years of age with exposure to adult HIV-positive and HIV-negative TB disease on residential plots between 1997 and 2007.

Results. During the study period the annual adult TB notification rate increased from 629 to 2 106/100 000 and the rate in children aged <15 years ranged between 664/100 000 and 1 044/100 000. The mean number of exposures to adult TB for TB-uninfected children, latently TB-infected children and TB cases were 5.1%, 5.4% and 33% per annum and the mean number of adult smear-positive cases per exposed child was 1.0, 1.6 and 1.9, respectively. Acquisition of TB infection was not associated with HIV status of the adult TB case to which the child was exposed, and 36% of child TB cases were diagnosed before the temporally closest adult case on their plot.

Conclusions. Childhood infection and disease were quantitatively linked to infectious adult TB prevalence in an immediate social network. Childhood infection should be monitored in high-burden settings as a marker of ongoing TB transmission. Improved knowledge of township childhood and adult social networks could also facilitate targeted active case finding, which may provide an adjunct to currently failing TB control strategies.

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Tuberculosis (TB) is a leading cause of mortality and morbidity globally. South Africa has the largest per capita TB burden in the world, and its TB notification rates are highest in crowded urban areas.[1,2] Although DOTS programmes (directly observed therapy, short course) have reduced TB prevalence and death rates in many regions of the world, implementation has not significantly impacted on TB transmission and incidence in Africa.[1] Adult TB disease comprises a combination of recent infections and reactivation of latent TB, and therefore represents both recent and past TB exposure. HIV co-infection increases the progression to disease of both recent and latent TB infection.[3,4] In contrast, childhood infection is acquired predominantly from adults, as child-to-child transmission is uncommon,[5] and young children with TB infection have a greater risk for progression to active disease than adults.[6] Infection and disease in young children are therefore a measure of recent TB transmission.

Country-specific TB rates in children are usually reported as smear-positive TB rates per 100 000 of the <15-year-old population.[1] This may substantially underestimate the true child TB burden due in part to the low proportion of smear-positive cases in children.[7] Furthermore, childhood TB disease occurs most frequently in children <2 years, resulting in the numerator of disease for this small age group being diluted by the larger population denominator (0 - 14-year-olds). This may explain why, despite significant increases in overall TB rates in the Africa region, the reported rates of childhood TB disease for South Africa have remained relatively unchanged since 2002.[1,8]

We have previously reported a growing TB epidemic in a peri-urban township heavily affected by HIV in Cape Town, South Africa. Recently urbanised, overcrowded, consisting largely of informal dwellings and with a population of low socio-economic status, the township is a well-demarcated, geographically isolated community. The HIV prevalence is 23% among adults[9] and 10% among adolescents aged 11 - 19 years.[10] The National TB Control Programme, based on WHO DOTS recommendations, appears to be functioning well at the community's single clinic, with treatment completion and cure

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rates of sputum-positive cases of approximately 80%[2] and case-finding rates of 67% in HIV-negative smear-positive patients.[9] However, local TB notification data show a rapid escalation in TB notification rates in adults. In particular the incidence rate of smear-positive TB increased from 326/100 000 in 1996 to 1 307/100 000 in 2005.[11,9] During the same period childhood TB notification rates have increased, but less markedly. We also reported a remarkably high TB prevalence rate of 26% in children aged 5 - 8 years, and a mean annual risk of TB infection in children of 4.1%.[12]

There are few data on TB exposure, infection and disease in children in a high TB and HIV disease burden setting. Adult TB notification data from this community and Geographic Information System (GIS) software were used to correlate the relationships between TB exposure, infection and disease in children with exposure to adult HIV-positive and HIV-negative TB disease on residential plots over time. The temporal association between adult cases and childhood disease was also explored.

Methods

The study community consists of a formal sector with demarcated, individually numbered serviced plots and an informal sector of shacks sharing communal services. Childhood TB disease cases from 1997 to 2007 were extracted from the community TB register. Childhood TB infection data were obtained from school-based tuberculin skin test (TST) surveys performed in the community in 2006 and 2007.[12]

The analysis was restricted to residents in the formal sector (approximately 78% of the community) who use the assigned plot number as their address; 1 - 20 houses are built on a single plot (average of 4 houses/plot). All adult TB cases notified by the community TB clinic between 1997 and 2007 were cross-referenced with both childhood TB cases and TST results from the school surveys. Children diagnosed with childhood TB disease during the study period were excluded from the TST database for this analysis. Adult TB cases were defined as patients ≥15 years of age and children as <15 years. Childhood TB infection was defined as an induration ≥10 mm in response to 2 units of purified protein derivative (PPD) administered intradermally.[12] Demographic and clinical data including age, gender, TB clinical diagnosis and HIV status were collected from the TB register and clinic records. These studies were approved by the University of Cape Town's Research Ethics Committee.

Data were analysed using STATA 9.0 (StataCorp, College Station, Texas). Bivariate analyses employed Student's *t*-test, Wilcoxon's sum rank test and the chi-square test, as appropriate. Rates of notified child disease were calculated using population denominators from community census data obtained in 1996, and biennially from 2002 to 2008 using linear interpolation. The time difference between adult and child cases was calculated based on the year of case diagnosis. Analysis of childhood TB cases initially included all adult TB exposures on the same residential plot, followed by an analysis of the adult TB exposure with the closest temporal link to each childhood TB case. Multiple logistic regression models were developed to determine characteristics of adult TB cases that were associated with positive TST results. As the age of the child was the only known factor associated with a positive TST result in the TST sample,[12] logistic regression models assessing factors associated with TST positivity included this variable and models were adjusted for clustering effect on plots. TB case characteristics were included as binary exposures (any exposure to a TB case with a particular characteristic) and as counts of exposures to a TB case with a particular characteristic, per child. All statistical tests were 2-sided at α=0.05. Average annual exposure rates for disease, infection and non-infection were calculated by dividing the percentage exposed to adult disease by the median age of the group of interest.

The ArcMap 9.2 (Esri™) Geographic Information System was used to assess the spatial distribution of childhood TB disease, infection and exposure with linked adult cases in the community.

Results

The population childhood TB notification rate varied between 315/100 000 and 1 105/100 000 over the study period, and adult TB rates increased from 629/100 000 to 2 106/100 000. Of the 1 708 notified TB cases 1 386 (81%) were resident in the formal sector. Of these 1 386 notifications 1 212 were adult and 171 were childhood cases, with 3 cases excluded from analysis because no age was recorded. TB cases in the formal sector did not differ from those in the informal sector in terms of age (p=0.21), gender (p=0.83), site of TB disease (i.e. pulmonary v. extrapulmonary disease (EPTB); p=0.60), HIV status among those tested (p=0.58) and outcomes of TB treatment (p=0.16). However, TB cases from the formal sector were more likely to be transferred out of the community than those from the informal areas (11% v. 6% respectively, p=0.016).

Childhood TB disease and exposure to adult TB at residence

The average annual rate of childhood TB disease in the formal sector of the community was 721/100 000 for all TB notifications and 65/100 000 for smear-positive TB. The median age of the 171 childhood TB cases was 2 years (interquartile range (IQR) 1 - 6 years), and 58% were female. In total 120 (70%) of cases were classified as primary TB, with 28 cases (16%) of pulmonary TB (PTB) and 23 cases (13%) of EPTB. The HIV testing was performed in 52 children (33 positive, 19 negative). Childhood TB cases occurred on 144 plots in the community, with a range of 1 - 3 cases per plot (mean 1.19 cases per plot).

Overall 113 (66%) of the childhood TB patients had one or more exposures to a notified adult TB case on their residential plot during their lifetime, with an annual exposure rate of 33%. In 28% of cases a family name was shared with one of the adults notified on the plot. In total 105 children (61%) were exposed to adult PTB cases, 91 being exposed to a mean of 1.9 smear-positive PTB cases and 40 to smear-negative PTB cases (53% of all child TB cases v. 23%, $p<0.001$). Fig. 1 shows the distribution of child TB cases and adult TB cases in the community. Children who were exposed to TB cases on their residential plot did not differ from those children not exposed in terms of age ($p=0.23$), gender ($p=0.51$) or HIV status ($p=0.76$). However, childhood TB cases not exposed on their residential plot were more likely to be retreatment TB cases than those exposed on the plot (12% v. 4%, $p=0.03$).

Fig. 1. Distribution of childhood TB cases and of notified adult TB cases from 1997 to 2007 in the community.

A total of 47 (42%) adult cases occurred in the same year as the child cases, 25 (22%) occurred before the child case and 41 (36%) occurred after the child case. When restricting the analysis to the adult TB case most closely linked temporally to each child case, the time interval between cases varied from 3 years before the child TB case to 8 years after the child TB case (Fig. 2, a). The disease characteristics of the closest adult TB cases by time are shown in Table I. The adult cases that occurred in the same year, before the child TB case and after the child case, did not differ from each other with respect to age ($p=0.91$), gender ($p=0.55$), HIV status ($p=0.87$) or treatment outcome ($p=0.40$). The time interval between diagnosis of all adult exposure cases and the child case ranged from 9 years before the diagnosis of the child TB case to 10 years after (median 1 year after child case; IQR 1 year before to 4 years after) (Fig. 2, b).

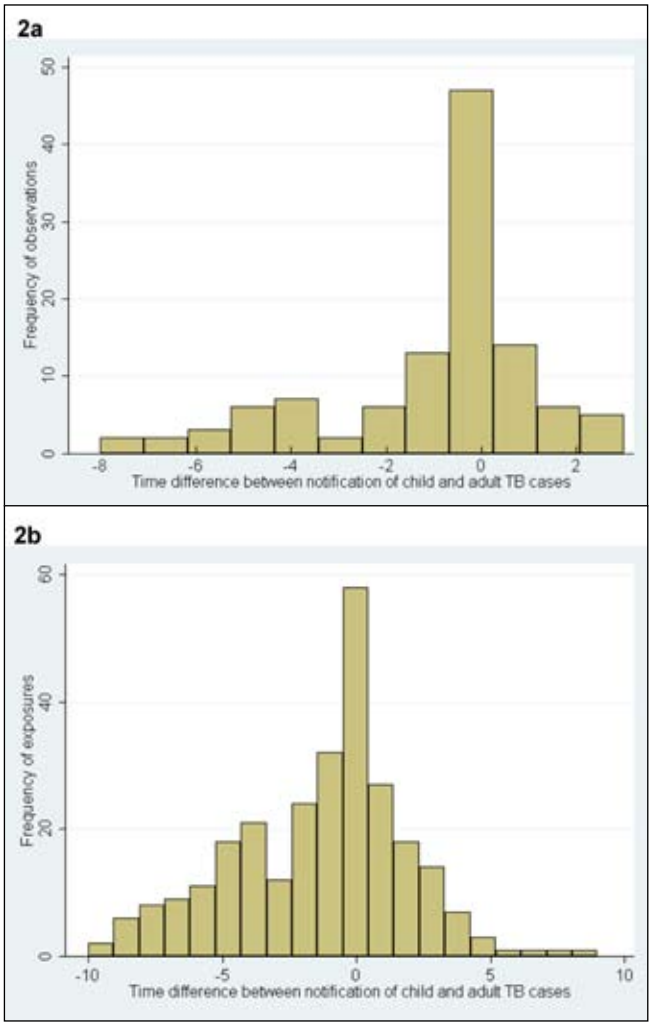

Fig. 2. Distribution of time difference between all adult TB exposures and childhood TB case (2a) and distribution of time difference between temporally closest adult TB exposure and childhood TB case on the same residential plot in study community (2b).

Childhood TB infection and exposure to adult TB at residence

Of the 831 children analysed in the TST survey, 651 (78%) lived in the formal sector and 640 (77%) of these had not had childhood TB. The subset of 640 children used in this study did not differ from the total TST study sample in terms of age ($p=0.95$), gender ($p=0.25$), BCG status ($p=1.00$) or positive TST reaction ($p=0.69$).

Of the 640 formal sector TST survey participants, 359 (56%) had been exposed to an adult TB case on their plot, and the proportion exposed was significantly higher among TST-positive compared with TST-negative children (65 v. 51%; $p=0.001$). The average annual exposure rate for children with infection was 5.4% per annum and for children without infection 5.1% per annum. Table II shows the distribution of type of adult TB case by TST result.

Table I. Temporal associations between childhood TB cases and exposure to temporally closest adult TB cases on the same residential plot

	Exposure to adult TB case (*N* (%))	Exposure to adult PTB case (*N* (%))	Exposure to adult smear-positive PTB case (*N* (%))	Exposure to adult smear-negative PTB case (*N* (%))
Child TB cases (*N*=171)	113 (66)	92 (53)	75 (44)	17 (10)
Adult TB case notified before or in same year as child case	72 (64)	60 (83)	48 (67)	10 (14)
Adult TB case notified after child case	41 (36)	32 (78)	27 (66)	5 (12)
p-value*	<0.001	0.47	0.93	0.80

*Comparison of exposure proportions between adult cases notified before or in same year as child, with those notified after child case.

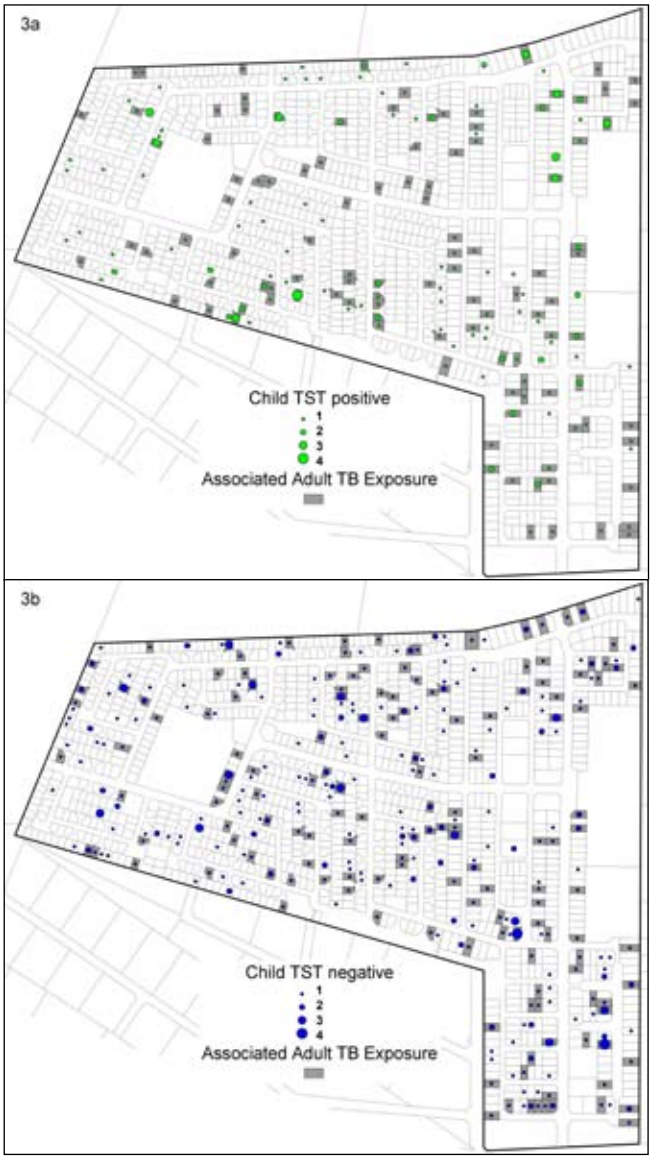

Fig. 3. Distribution of TST positive (a) and TST negative (b) results and of adult TB cases from 1997 to 2007 in the community.

Fig. 3 (a and b) show the distribution of adult TB cases with TST-positive and TST-negative results, respectively. The odds of a positive TST result was 71% higher in those children exposed to any TB case on their plot (odds ratio (OR) 1.71; 95% confidence interval (CI) 1.20 - 2.44), 88% higher in those exposed to PTB (OR 1.88, 95% CI 1.32 - 2.68), and 110% higher in those exposed to smear-positive PTB disease (OR 2.10, 95% CI 1.46 - 3.01) compared with no plot-based exposure to adult TB. Exposure to smear-negative TB was not associated with a positive TST result (OR 0.95, 95% CI 0.59 - 1.54), and this finding persisted after adjusting for HIV status of notified adult TB cases (OR 0.68, 95% CI 0.21 - 2.18).

Multiple exposures increased risk further, and the odds of a positive TST result increased by 60% for every additional exposure to a smear-positive PTB adult (OR 1.60, 95% CI 1.21 - 2.11). The mean number of adult smear-positive exposures for TST-positive children was significantly higher than for TST-negative children (1.6 v. 1.0, $p<0.001$).

Discussion

We have shown that children in this community have extremely high exposure to adult TB on their residential plot. The strongest association of childhood TB infection and disease was with adult cases of smear-positive PTB rather than smear-negative PTB or EPTB. There was no independent risk of infection associated with exposure to adults with HIV-associated TB, in keeping with the previous finding in this community that child TB notification rates have not increased parallel with adult HIV/TB notification rates.[12] It is therefore probable that the HIV epidemic may indirectly impact on childhood TB disease rates by increasing the burden of adult smear-positive TB cases in children's immediate social network.[11]

While TST surveys can assess rates of TB infection in children, our residential plot analysis was unique in allowing an assessment of annual rate of exposure to adult TB cases. The average annual exposure to adult TB case on residential plots was high for all children living on serviced plots in this community (5.1%), but was extremely high for children with TB disease (33% per annum). These exposure rates probably drive the high rates of child TB infection and disease in this community. By age of 6 years, at school entry, approximately

Table II. Tuberculin skin test results and exposure to adult TB cases on the same residential plot

TST	Exposure to adult TB case (*N* (%))	Exposure to adult PTB case (*N* (%))	Exposure to adult smear-positive PTB case (*N* (%))	Exposure to adult smear-negative PTB case (*N* (%))
Total (*N*=640)	359 (56)	316 (49)	265 (41)	115 (18)
TST positive (*N*=231)	149 (65)	137 (59)	123 (53)	39 (17)
TST negative (*N*=409)	210 (51)	178 (44)	142 (35)	76 (19)
p-value (TST +ve v. TST –ve)	0.001	0.001	<0.001	0.61

26% of children are latently infected with TB[12] and most adolescents are infected before sexual debut.[12,10] The average annual TB notification rate for children under 15 years in this community was 721/100 000 and their smear-positive rates were nearly 3-fold higher than the South African national rates (83 v. 30/100 000).[1] The very high rate of HIV-related TB in young adulthood may also result from the high proportion of young adolescents latently infected with TB.

The residential plots contain multiple households, which share communal water and sanitation services and constitute a geographically defined unit for social interaction between adults and children outside the immediate household. Family names differed between children and all their adult exposures in 72% of cases.

This study was restricted to the formally serviced sector in which 78% of the community is resident. We could not define the geographical area of interaction between adults and children living in the informal sectors, and shack identifiers in this sector were not constant over the study period. For this analysis we also assumed that the children had all lived in the community since 1997 or from birth. Some children may have moved into the community subsequent to these dates; however, this would have decreased the strength of association between adult and child TB exposures and resulted in an under-estimation, rather than over-estimation, of the contact rate between adult and children with TB infection and disease.

The majority of childhood TB disease (64%) was diagnosed in the same year or soon after an index adult TB case was notified to the TB control programme. However, over a third of child TB cases in this study were notified before the temporally closest notified adult case on their plot. Adult cases were diagnosed as much as 8 years after the childhood case of TB. These more temporally distant cases probably represent secondary or even tertiary cases due to ongoing adult-to-adult transmission. Potential explanations for this delayed adult presentation include a primary source that may have remained subclinical, who subsequently moved off the plot, or died before diagnosis. However, these data illustrate a significant prevalence of undetected and untreated adult TB on these plots.

Molecular epidemiology from this community shows that adult-to-adult transmission occurs predominantly outside the residential plot.[13] The use of molecular epidemiology among children is complicated by the low frequency of smear- and culture-negative disease. However, we have demonstrated that child TB infection and disease rates are strongly associated with exposure to adult TB within their restricted social environment as defined by their residential plot. The high rates of child TB infection and disease therefore indicate failure to control prevalent infectious adult cases. To reduce TB exposure, enhanced and active case finding (ACF) must be combined with the DOTS-based programme. The yield of ACF will vary in different settings. A random, community-based survey in this community showed that 0.1% of the adult population had undiagnosed smear-positive TB and an equal proportion had undiagnosed culture-positive TB, similar to the range of 0.1 - 0.2% in case finding surveys in other developing countries.[14,15] We postulate that ACF targeted at residential plots, rather than only households, may have a higher yield than these cross-sectional surveys and may decrease the prevalence of infectious cases, resulting in a lower rate of transmission to children. In addition to the TB case detection yield, efficacy of such a targeted intervention over time could be assessed through repeated TST surveys in children, to ascertain the impact on childhood infection rates.

In conclusion, TB transmission within households and close social networks is an important component of both childhood infection and disease in this community. Childhood TB is a sentinel of infectious adult prevalence and therefore childhood infection and disease rates need to be monitored in these high prevalence settings in order to ascertain the true burden of infection and disease. Targeted ACF, aimed at close social networks of child TB cases may be an appropriate intervention in this setting.

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Potential conflicts of interest. All authors no conflicts.

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Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa

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SUMMARY

SETTING: Crowded townships of Cape Town, South Africa, where human immunodeficiency virus (HIV) prevalence and tuberculosis (TB) notification rates are among the highest in the world.

OBJECTIVES: To determine age-specific prevalence rates of latent tuberculosis infection (LTBI) among HIV-negative individuals, and the annual risk and force of infection during childhood and adolescence.

DESIGN: A cross-sectional survey using a standardised tuberculin skin test (TST) in HIV-negative individuals aged 5–40 years. A TST diameter of ⩾10 mm was defined as indicative of LTBI.

RESULTS: Among 1061 individuals, only 4.7% had low-grade TST responses of 1–9 mm. However, the proportions of individuals with TST ⩾10 mm increased from 28.0% in the 5–10 year age stratum to 88.0% in the 31–35 year age stratum. The mean annual risk of infection was 3.9% up to 5 years of age. The estimated force of infection (the rate of acquisition of LTBI among the residual pool of non-infected individuals) increased throughout childhood to a maximum of 7.9% per year at age 15 years.

CONCLUSIONS: Extremely high rates of infection in childhood and adolescence result in very high LTBI prevalence rates in young adults who are most at risk of acquiring HIV infection. This may be an important factor fuelling the high rates of HIV-associated TB in southern Africa.

KEY WORDS: TB infection; age; sex; Africa; TST

SOUTH AFRICAN tuberculosis (TB) notifications have increased six-fold over the last two decades, largely as a result of increasing human immunodeficiency virus (HIV) prevalence.[1] A total of 461 000 new cases of TB in 2007 reflected one of the highest national TB notification rates in the world. The overall incidence rate was estimated at 948 per 100 000 population, of which 73% were estimated to be co-infected with HIV. South Africa alone accounted for approximately 25% of the global burden of HIV-associated TB.[1]

Total TB notifications in Cape Town, a city of 3.2 million people, reached 27 000 in 2006.[2] The distribution of TB cases within this population, however, is very unequal, with unprecedentedly high burdens in the crowded and socially deprived African townships. Here TB annual notification rates were reported to exceed 1500/100 000 in 2006.[3–5] Fuelled by high HIV prevalence, TB notifications are now most frequent between the ages of 20 and 40 years.[6]

This marked deterioration of TB control in South Africa over the past two decades of increasing HIV prevalence has occurred despite reported progress towards National TB Control Programme case management targets.[1,7] Coverage using the World Health Organization (WHO) DOTS strategy is 100% and case detection rates have remained above target since 2003.[1] In the decade from 1996 to 2005, treatment default and treatment failure during treatment improved respectively from 18.1% to 10.4% and from 3.5% to 1.7%.[8]

In 1990, Karel Styblo, a leading protagonist of effective case management for TB control, postulated that 'the impact of HIV infection on the epidemiological situation of TB is so large that under some conditions, the tools available at present for TB control

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will fail to restrain the incidence of TB caused by HIV infection'.[9] He further proposed that the impact of HIV infection would depend not only on the prevalence of HIV infection but also on the prevalence of latent TB infection (LTBI) and the annual risk of TB infection (ARTI) in the general population. However, although the HIV epidemic in South Africa has been carefully monitored by annual seroprevalence surveys among antenatal women and by household surveys,[7,10] few data describe the prevalence of LTBI or the ARTI in the general population.

We therefore undertook a study to measure the prevalence of LTBI in the township populations of Cape Town. The tuberculin skin test (TST) has been the most widely used of all the immunological tests for estimation of prevalence, incidence and trend of *Mycobacterium tuberculosis* infection in populations, despite concerns about its sensitivity and specificity.[11] We assessed TST responses among healthy HIV-negative residents of high-density townships aged between 5 and 40 years and used these to derive estimates of the age-specific prevalence of LTBI. Further analyses estimated the ARTI in the study population and the force of TB infection (the rate of acquisition of TB infection among the residual pool of non-infected individuals). These data provide important insights into the explosive impact of the high rates of HIV acquisition in young adults on TB control.

METHODS

Study population

TST responses were assessed in 1061 healthy individuals, including children and adolescents aged 5–17 years (n = 832) and HIV-negative adults aged 18–40 years (n = 229). Participants were residents of high-density predominantly black townships of Cape Town, where annual TB notification rates in 2006 exceeded 1500/100 000.[3–5] Children were all school attendees, as described in a previous study,[3] and healthy HIV-negative adults were recruited from a prospective cohort recruited for a Phase III HIV vaccine study (n = 60) or from HIV voluntary counselling and testing centres (n = 169). Eligibility criteria included residence within the study communities described above, age between 5 and 40 years, confirmed HIV-negative (adults only), non-pregnant, no previous or currently suspected episodes of TB and no exposure to isoniazid preventive therapy or corticosteroids.

These studies were approved by the University of Cape Town Research Ethics Committee.

Tuberculin skin testing

TST responses were assessed using the WHO-recommended standard methodology.[12] Two tuberculin units of purified protein derivative (PPD) RT23 (Statens Serum Institut, Copenhagen, Denmark) were administered intradermally to the volar surface of the left forearm. Induration was assessed between 48 h and 72 h after inoculation and the diameter was measured. Age and sex were also recorded for each participant, as was bacille Calmette-Guérin (BCG) scar status in children.

Definition of latent TB infection

TST induration of ⩾10 mm at the inoculation site was considered indicative of LTBI.

Statistical analysis

'R' statistical software was used for analyses. The relationship between TST diameter and age was explored by regression analysis. A generalised non-parametric logistic model[13] was proposed to quantify the relationship between LTBI prevalence rate (as defined by TST diameter ⩾10 mm) and age. We fitted the model using the penalised regression spline approach.[14] To evaluate sex differences, prevalence rates for female and male subjects were obtained separately using a generalised semi-parametric logistic model which was then fitted to the data.[15] The proportional rate of change in TB prevalence per year increase in age was estimated using the derivative of the prevalence rate with respect to the age of subjects. The annual risk of infection was calculated as: $1 - (1 - \text{prevalence})^{1/\text{mean age}+0.5}$. The force of infection was also calculated at specific ages for the pool of individuals who remained non-infected (annual change in prevalence/[1 − prevalence]).

RESULTS

TST diameters

TST responses were available from 1061 individuals, 524 male and 537 female. Diameters of induration ranged between 0 and 35 mm, with a mode within the 16–20 mm stratum (Figure 1). No TST response was recorded in 535 individuals (50.4%), 1–5 mm in

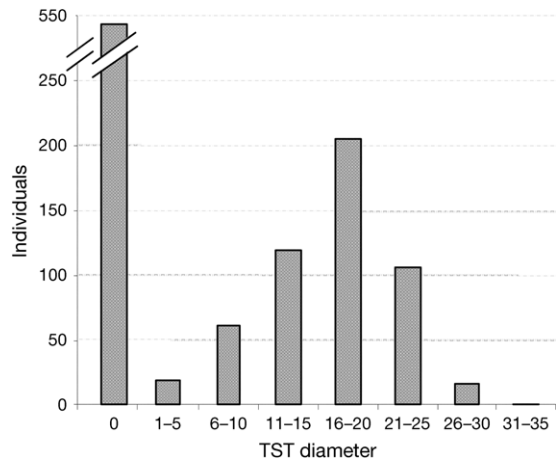

Figure 1 Frequency distribution of diameters of induration at 48–72 h after standardised TST in 1061 individuals aged 5–40 years. TST = tuberculin skin test.

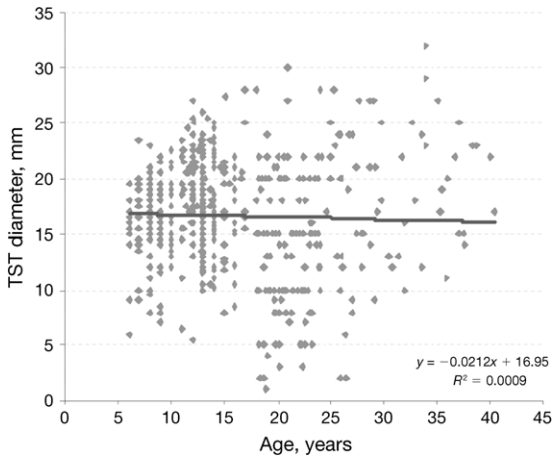

Figure 2 Scatter plot of diameters of TST induration vs. age at time of performing test. Linear regression demonstrates no significant relationship between size of TST response and age. TST = tuberculin skin test.

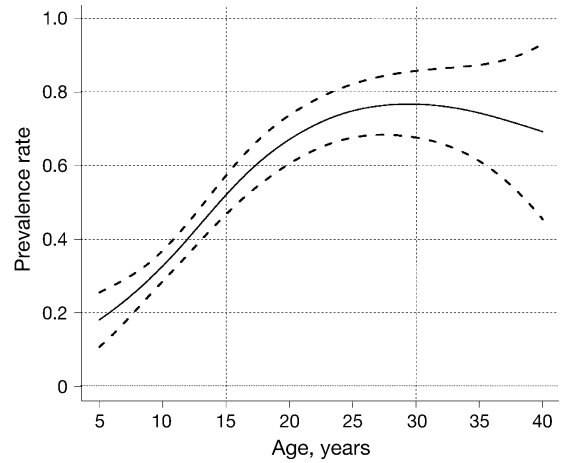

Figure 3 Relationship between the prevalence of TST diameters of ⩾10 mm and age for 1061 subjects, using a generalised non-parametric logistic model.[13] The 95% point-wise confidence bands are also plotted. The prevalence rate reached a maximum of 82% at an age of 27.5 years. TST = tuberculin skin test.

8 individuals (0.8%), 5–9 mm in 41 individuals (3.9%) and ⩾10 mm in 477 (45%) individuals (Figure 1).

To establish whether the magnitude of TST responses was associated with age, TST diameters recorded as ⩾1 mm were plotted against age (Figure 2). The linear regression line for these data approximated to a horizontal line, indicating no overall significant relationship. Furthermore, as previously reported,[3] there was no significant difference in mean TST diameter among those aged 5–17 years when comparing those who had observable BCG scars (n = 213) and those who did not (P = 0.28).

Prevalence of LTBI

Using a TST diameter of ⩾10 mm to define LTBI, the age-stratified prevalence of infection for the total population and for male and female sub-populations was calculated (Table 1). LTBI prevalence increased steadily from 28.0% in the 5–10 years age stratum to a peak of 88.2% in the 31–35 years age stratum.

To better demonstrate the quantitative relationship between LTBI prevalence and age, a non-parametric curve fitting approach was used (Figure 3). The smoothed curve shows best fit of the prevalence with increasing age together with 95% confidence intervals (95%CIs). This analysis was also used to derive estimates of the LTBI prevalence at specific ages (Table 2). This shows that LTBI was estimated to be present in approximately one third of 10-year-old children, one half of adolescents aged 15 years, two thirds of 20-year-olds and three quarters of 25-year-olds (Table 2).

LTBI prevalence among males and females

Estimates of LTBI prevalence were separately derived for male and female subjects (Figure 4). The difference in prevalence rates between the male and female subjects was greatest among the older study participants. The maximum estimated LTBI prevalence among males was at 33 years (85%) compared to

Table 1 Prevalence of positive TST diameter of induration (⩾10 mm) stratified by age in township residents (n = 1061) of Cape Town, South Africa

Age category, years	Mean age, years	Participants n	TST ⩾10 mm Patients n	TST ⩾10 mm Prevalence % (95%CI)
5–10	8.3	325	91	28.0 (24.1–32.3)
11–15	12.7	451	188	41.7 (37.2–46.3)
16–20	17.3	102	67	65.7 (56.0–74.2)
21–25	22.3	103	71	68.9 (59.4–77.1)
26–30	27.4	42	32	76.2 (61.3–86.7)
31–35	32.7	17	15	88.2 (64.4–98.0)
36–40	37.5	20	12	60.0 (38.6–78.2)
All	14.1	1061	477	45.0 (42.0–48.0)

TST = tuberculin skin test; CI = confidence interval.

Table 2 Estimated prevalence (95%CIs) of LTBI* at specific ages generated using a generalised non-parametric logistic model[13]

Age, years	Estimated age-specific LTBI prevalence % (95%CI)	Mean annual rate of TB infection (95%CI)	Force of infection [Δ prevalence/(1 − prevalence)] (95%CI)
5	18.1 (10.7–25.5)	3.9 (2.2–5.7)	3.0 (0.8–5.2)
10	32.7 (28.6–36.8)	3.9 (3.3–4.5)	5.2 (2.7–7.6)
15	52.0 (46.7–57.3)	4.8 (4.1–5.5)	7.9 (2.5–13.2)
20	67.1 (60.4–73.8)	NA	6.8 (−1.7–15.2)
25	74.9 (67.6–82.2)	NA	3.6 (−8.5–15.7)
30	76.7 (67.7–85.7)	NA	—
35	74.3 (61.2–87.4)	NA	—
40	69.3 (45.4–93.2)	NA	—

*LTBI is defined as a tuberculin skin test diameter of induration ⩾10 mm. The estimated force of TB infection (the annual change in prevalence among the remaining non-infected pool of individuals = Δ prevalence/[1 − prevalence]) and the mean annual risk of infection for children aged 5–15 years are shown with 95%CIs.
LTBI = latent TB infection; TB = tuberculosis; CI = confidence interval; NA = not applicable.

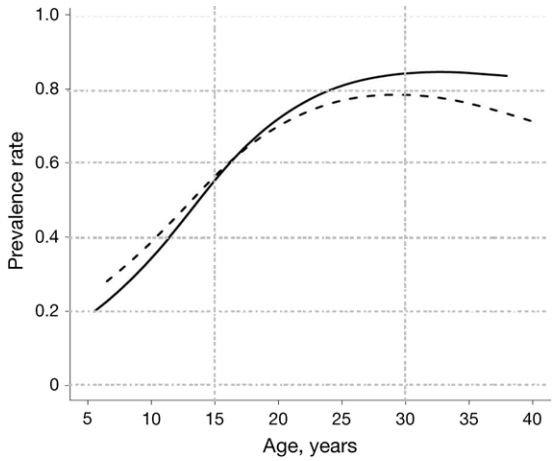

Figure 4 Fitted prevalence rate vs. age for male (solid line) and female (dotted line) subjects, respectively, using the generalised non-parametric logistic model.[13] The interaction term of age and sex was also considered in the model. The prevalence rate reached a maximum (points indicated) of 92% at age 31.5 years for male and the peak 84% at age 26.5 years for females.

29 years among females (78%). The decreasing trend in prevalence at ages >29 years was mainly due to changes in prevalence among females. However, the main sex effect ($P = 0.22$) and the age-sex interaction effect ($P = 0.29$) were not statistically significant.

Rates of change in LTBI prevalence by age

Estimates of the change in LTBI prevalence rate per year increment in age were derived for males and females (Figure 5). The patterns of the curves were similar for both groups. The rate of change in prevalence rate increased from 5 years of age and peaked at the age of 13 years. Maximum rates were 4.4% per year in males and 3.7% per year in females (Figure 5). After age 13 years, the annual rate of change in LTBI prevalence decreased.

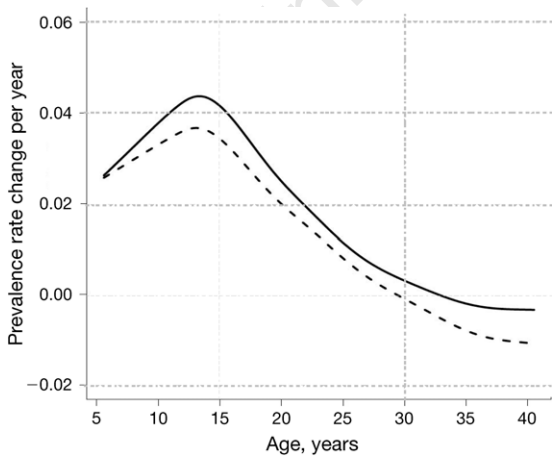

Figure 5 The annual change in the prevalence of TST diameters of ⩾10 mm vs. age for male (solid line) and females (dotted line) subjects are plotted using a generalised non-parametric logistic model.[13] The change in the prevalence of TST responses ⩾10 mm increased between age 5 and age 13 years, and reached a maximum at age 13 years in both groups. TST = tuberculin skin test.

Annual risk and force of infection

Further analyses estimated the ARTI among children aged 5, 10 and 15 years, showing a range of 3.9% to 4.8% per year (Table 2). The force of infection was also calculated at specific ages for the pool of individuals who remained non-infected (annual change in prevalence/[1 − prevalence]; Table 2). This parameter reached a peak of 7.9% (95%CI 2.5–13.2) per year among individuals aged 15 years and was negative above the age of 30 years. This analysis of force of infection did not adjust for active TB disease (an exclusion criterion from the cohort) or TB-associated mortality.

DISCUSSION

We have shown rapidly increasing prevalence of TST responses in healthy HIV-negative township residents aged between 5 and 40 years. Using a cut-off of ⩾10 mm diameter of induration as evidence of LTBI, we found that, by the age of school entry, almost a fifth of children were already infected. By the average age of sexual debut at 15 years,[16] 50% of adolescents in these communities were infected. By the age of 25 years, when HIV prevalence peaks in South Africa,[7] approximately 75% of individuals had evidence of LTBI. The rate of increase in the prevalence of LTBI was maximal at 13 years of age in both males and females. Between the ages of 5 and 15 years, the mean ARTI remained exceptionally high (range 3.9–4.8%), while the force of infection (the risk of infection in the residual pool of non-infected individuals) was maximal, at 7.8%, at the age of 15 years.

The complexities and limitations of the TST have been extensively discussed elsewhere.[11,12,17] Controversies have included the choice of the antigen utilised, the threshold for defining positivity and the performance characteristics of the test in different settings. The tuberculin reagent employed in this study was standard WHO-recommended PPD RT23, which allows comparison with other population surveys.[18] Non-specific sensitivity resulting from exposure to environmental mycobacteria impairs test performance in some populations,[11] manifesting with a high proportion of low-positive results. In our study population, there was a relatively clear separation between negative and positive results; the mode of positive results was in the 16–20 mm diameter stratum and the proportion of low positive results was very small. Furthermore, diameter of induration was not directly associated with known confounders, such as presence of BCG scar or the age of participants. We therefore used the conventional cut-off for test positivity of ⩾10 mm.

The relationship between prevalence of LTBI, age and sex is determined by the individual's current and lifetime exposure to infection. While prevalence in children reflects more recent transmission, prevalence in adults reflects overall historical trends in transmission risk. It has been observed that, at low prevalence rates of LTBI, instability of TST reactions may lead to false interpretations of secular trends.[19] However, the predictive value of TST responses is much greater when prevalence of infection is high.[11] In our study population, TST performance characteristics appeared good and LTBI prevalence rates were exceptionally high. Furthermore, trends in sputum smear-positive TB notifications are very well documented, increasing relentlessly over the last two decades in South Africa and particularly in the study communities.[1,6,20]

To explore the relationship between LTBI prevalence, age and sex, we fitted a generalised nonparametric (i.e., no assumptions about the data distribution) logistic model. The resultant 'S'-shaped curve had strong similarities with age and sex prevalence curves from TST surveys performed over 50 years ago in urban and rural sites of Bechuanaland (Botswana), Ghana, Liberia, Nigeria, Sierra Leone and Swaziland.[21]

Between 5 and 15 years of age, the curve is concave upwards (Figure 3). This could be indicative of decreasing transmission in recent years[22] but, in the known context of rapidly increasing TB notifications over the past two decades, this is much more likely to be indicative of increasing risk of infection with age.[23] The finding of maximal risk of acquisition in the mid-teenage years may reflect social mixing patterns and associated TB exposure in this age group.[23,24]

From 15 to 30 years, the curve had an exponential form (Figure 3), compatible with either a steady or decreasing infection risk with age.[23] From 30 years onwards, declining prevalence with age was more marked in females. Declining of immunological memory with age did not appear to be a significant contributor, as reaction size was not shown to diminish significantly with age. Alternative explanations could include immigration from lower prevalence settings, survival from a period of lower TB transmission or preferential progression of individuals with TST positivity to TB disease, as a history of TB treatment was an exclusion criterion in adult participants.

Larger TST studies, such as those performed between 1955 and 1960, included heterogeneous populations with very variable TB exposure rates.[23] This study, which was modest in size (n = 1061), focused particularly on a population within a single city that has an extraordinary high burden of HIV and TB disease. The study population was not representative of the total community, as HIV-infected individuals and those who had already developed TB disease were excluded. We believe that the risk of TB infection and performance characteristics of TST in HIV-infected patients and patients with TB disease may be very different. The results are therefore only descriptive of those remaining free of both TB and HIV. The number of individuals aged >30 years was relatively small, leading to wide CIs around prevalence estimates in this age group. The differences between calculated and modelled prevalence estimates (Table 1 and Figure 3) were not statistically significant.

The mean ARTI in children aged <5 years was approximately 4%, and while the more dynamic measure of force of infection at a specific age was similar at 5 years, it reached a peak of 7.9% at age 15 years. Such rates of TB infection, as reported elsewhere in Cape Town,[25] are unprecedented in the modern post-chemotherapy era. The calculation of force of infection takes into account both the prevailing rate of infection as well as historical exposure, and may tend to underestimate the force of infection in the context of rapidly increasing TB notification rates, as has occurred in these communities. The high ARTI suggests that individuals may be recurrently exposed to *M. tuberculosis*. This may result in infection with multiple strains, and also potentially explains the observed high rates of TB recurrence in individuals receiving curative TB treatment.[6]

In summary, we have shown an extremely high rate of acquisition of LTBI in childhood and adolescence in poor African townships of Cape Town. The rates observed are as high as the highest rates observed in the WHO surveys conducted in African countries over 50 years ago, in the era prior to modern multidrug TB treatment and TB control programmes.[21] The combination of high prevalence and force of infection in adolescence before the acquisition of HIV infection may be a key factor underlying the explosive HIV-associated TB epidemic in South Africa. HIV prevalence among those aged 20–39 years in these communities reached 30% in 2002,[6] and the current data suggest that approximately two thirds of these individuals were likely to have already been infected with *M. tuberculosis* infection prior to HIV acquisition. This may be an important factor fuelling high rates of HIV-associated TB in southern Africa.

The long-term aim of TB control is to lower infection rates in successive generations. Present facility-based TB control is failing to reduce TB infection rates in children and adolescents in these communities. Systematic evaluation and reduction of infection rates in these high-burden communities of Southern Africa should be incorporated as a target of TB control.

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RÉSUMÉ

CONTEXTE : Les faubourgs peuplés de Cape Town, Afrique du Sud, où les taux de déclaration de la prévalence du virus de l'immunodéficience humaine (VIH) et de la tuberculose (TB) sont parmi les plus élevés du monde.

OBJECTIF : Déterminer les taux de prévalence de l'infection tuberculeuse latente (LTBI) spécifiques pour l'âge parmi les individus séronégatifs pour le VIH et le risque annuel et la puissance de l'infection au cours de l'enfance et de l'adolescence.

SCHÉMA : Enquête transversale utilisant un test tuberculinique standardisé (TST) chez les individus séronégatifs pour le VIH âgés de 5 à 40 ans. On a défini comme indiquant la LTBI un diamètre du TST ⩾ 10 mm.

RÉSULTATS : Parmi 1061 individus, 4,7% seulement ont eu des réactions du TST de faible degré (1 à 9 mm). Toutefois, les proportions d'individus dont les diamètres de TST sont ⩾10 mm augmentent de 28% dans la classe d'âge de 5 à 10 ans jusqu'à 88% dans la classe d'âge de 31 à 35 ans. Le risque annuel moyen d'infection est de 3,9% jusqu'à l'âge de 5 ans. La puissance estimée de l'infection (taux d'acquisition de la LTBI dans le réservoir résiduel d'individus non-infectés) augmente pendant l'enfance jusqu'à un maximum de 7,9% par an à l'âge de 15 ans.

CONCLUSIONS : Les taux extrêmement élevés d'infection dans l'enfance et l'adolescence entraînent les taux très élevés de prévalence de la LTBI chez les jeunes adultes où le risque de l'infection VIH est maximal. Ceci peut être un facteur important qui nourrit les taux élevés de tuberculose associée au VIH en Afrique du Sud.

RESUMEN

MARCO DE REFERENCIA: La prevalencia de infección por el virus de la inmunodeficiencia humana (VIH) y las tasas de notificación de tuberculosis (TB) en las localidades densamente pobladas de la Ciudad del Cabo en Sudáfrica, se encuentran entre las más altas del mundo.

OBJETIVOS: Determinar las tasas de prevalencia específicas por edad de infección tuberculosa latente (LTBI) en las personas con examen serológico negativo para el VIH y evaluar el riesgo anual y la fuerza de la infección durante la niñez y la adolescencia.

MÉTODOS: Se llevó a cabo un estudio transversal aplicando la prueba cutánea de la tuberculina (TST) a personas entre los 5 y los 40 años de edad, con serología negativa para el VIH. Se escogió una reacción de diámetro ⩾10 mm como indicativo de LTBI.

RESULTADOS: De las 1061 personas que participaron, solo 4,7% tuvieron reacciones menores a la TST (con diámetros de 1 mm a 9 mm). Sin embargo, la proporción de personas con reacciones de diámetro ⩾10 mm aumentó de 28,0% en el grupo de 5 a 10 años de edad hasta 88,0% en el grupo de 31 a 35 años de edad. El riesgo anual de infección promedio fue 3,9% hasta los 5 años de edad. La fuerza calculada de la infección (la tasa de contracción de la LTBI en un grupo residual de personas sin infección) aumentó durante la infancia, hasta un máximo de 7,9% por año a la edad de 15 años.

CONCLUSIONES: Las tasas extremadamente altas de infección tuberculosa en la infancia y la adolescencia conllevan altas tasas de prevalencia de LTBI en los adultos jóvenes, quienes presentan el mayor riesgo de contraer la infección por el VIH. Este puede ser un factor importante que alimenta las altas tasas de tuberculosis asociada con la infección por el VIH en Sudáfrica.

MAJOR ARTICLE

Tuberculosis Transmission to Young Children in a South African Community: Modeling Household and Community Infection Risks

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Background. Tuberculosis transmission is determined by contact between infectious and susceptible individuals. A recent study reported a 4% annual risk of child tuberculosis infection in a southern African township. A model was used to explore the interactions between prevalence of adult tuberculosis infection, adult-to-child contacts, and household ventilation, which could result in such a high annual risk of tuberculosis infection.

Methods. Number of residents per household and tuberculosis incidence were derived from a household census and community tuberculosis registers. Using the Wells-Riley equation and probability analyses of contact between infectious adults with tuberculosis and preschool children, we estimated the annual risk of tuberculosis infection within and outside of the home.

Results. There was a mean of 2.2 adults per child-containing household with a 1.35% annual adult smear-positive tuberculosis notification rate. The maximal household annual risk of tuberculosis infection was 3%, which was primarily determined by the number of resident adults. Transmission risk outside the home increased with increasing number of households visited. Transmission probabilities were sensitive to exposure time, ventilation, and period of adult infectivity. The benefits of increased ventilation were greatest when the period of infectivity was reduced. Similar reductions in household transmission could be achieved by increasing ventilation from 2 to 6 air changes/hour or by separating child and adult sleeping areas.

Conclusions. The annual risk of tuberculosis infection of preschool children predominantly results from infectious residents in the home. However, even with limited social interactions, a substantial proportion of transmissions may occur from nonresident adults. The benefits of increased ventilation are maximized when the period of infectivity is reduced by prompt treatment of source cases.

South Africa is now the country with the fifth highest tuberculosis burden in the world, with high rates of both adult and childhood (ages 0–15 years) tuberculosis notifications [1]. The total number of tuberculosis notifications in the South African city of Cape Town, with 3.2 million people, reached 27,000 in 2006 [2]. However, the distribution of tuberculosis cases within the city is very unequal, with unprecedented high burdens in the crowded and socially deprived African townships. In these townships, housing consists largely of informal shack dwellings, in which the annual tuberculosis notification rates exceed 1500 per 100,000 persons [3–5]. Whereas adult tuberculosis disease is caused by a combination of reactivation of remote infection and rapid progression of recent adult-to-adult transmission [6], childhood disease reflects rapid progression from recent adult-to-child transmission [7]. Childhood (ages 0–15 years) tuberculosis notification rates have been reported to be 3.5 times the adult rate in specific highly burdened Cape Town communities, where childhood tuberculosis contributed 39% of the total tuberculosis case load [8]. Recent studies of childhood tuberculosis infection rates in southern Africa townships have reported annual risks

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Table 1. Age Distribution of Residents in All Study Site Households, Households with Adults Residents Only, and Households with Children Aged <15 Years or <5 Years

Households	No. of households	No. of children aged <5 years	No. of children aged 5–15 years	No. of adults	No. of adults per household	Total no. of residents	No. of residents per household
All households	6654	1051	1640	12,097	1.82	14,788	2.22
Households with only adults	4946	0	0	8148	1.65	8148	1.65
Households with children aged <15 years	1708	1051	1640	3949	2.31	6640	3.89
Households with children aged <5 years	918	1051	516	2083	2.27	3650	3.98

of tuberculosis infection as high as 4% per annum [9–11]. This annual risk of tuberculosis infection is similar to reported values from 60 years ago, before implementation of national tuberculosis control programs [12].

Childhood tuberculosis infection is quantitatively related to exposure of susceptible children to adults who have sputum smear–positive tuberculosis [13, 14]. The prevalence of infectious adults is determined by the annual incidence rate of smear-positive tuberculosis in adults and the mean time of infectivity, the period between becoming infective and either initiation of effective therapy or death. The prevalence of untreated tuberculosis is therefore primarily determined by the effectiveness of the tuberculosis control program to identify, diagnose, and effectively treat infective tuberculosis cases. The risk of a possible transmission event is related to the number of contacts a child has with infectious adults. The efficiency of transmission, in turn, is determined by the infectiousness of the source, the length of contact, and the environmental characteristics at the site of a contact. Tuberculosis transmission thus results from the interplay between social interactions, environmental factors, and the prevalence of infective adults. The period of infectiousness (Δ) of adults is the only modeled parameter affected by the activities of the tuberculosis control program.

We modeled tuberculosis transmission among preschool children (aged 0–5 years), both in and outside of their primary residence, using the distribution of resident adults per household and the prevalence of adult infectious tuberculosis. The modeled transmission probabilities were adjusted for length of exposure time and variable household ventilation characteristics. We also explored decreased periods of adult infectivity and increased household ventilation, which would be required to achieve significant reductions in transmission.

METHODS

Study design. The study aim was to explore probabilities of transmission from adults to preschool children within and outside of households in a South African township. The Wells-Riley equation is a well-known transmission model that has been used to describe airborne transmission probabilities of a single enclosed room or space with defined ventilation [14]. The Wells-Riley equation, which has been applied to a wide range of transmission scenarios [15–19], was used in combination with the distribution of adults per household and their probability of being infectious, to explore adult-to-child tuberculosis transmission probabilities.

Study community. The study site used to provide data inputs to these modeling analyses is a periurban township (Site-M) near Cape Town, South Africa, which was established in 1992 and has grown to a 2008 population of 14,788 people. The township is home to an almost exclusively African population, the adult human immunodeficiency virus (HIV) prevalence in 2005 was 23%, and the majority of persons have low socioeconomic status [3]. Unemployment exceeds 50%, and housing predominately consists of closely aggregated, formal and informal structures. The township has clearly demarcated boundaries and constitutes a well-defined population for research studies and community health interventions.

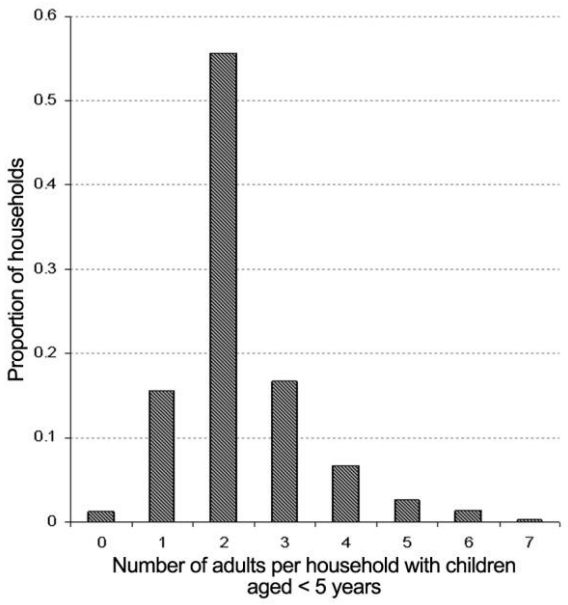

Figure 1. The proportions of households with preschool children (aged <5 years) in which different numbers of adults are resident. A total of 2083 adults and 1051 preschool children were resident in 918 households. Data were derived from a 2008 household survey performed at the study site.

Table 2. Tuberculosis Notifications at the Study Site Reported in 2004–2008, Stratified by Age

Measure	Total population	Adults aged >15 years	Children aged 5–15 years	Children aged <5 years	Adults with smear-positive tuberculosis
No. of tuberculosis notifications	1289	1158	45	86	670
Population years of exposure	67,747	53,056	9181	5510	53,056
Tuberculosis rate per 100,000 population (95% CI)	1909 (1799–2018)	2137 (1984–2339)	546 (346–546)	1522 (1419–1533)	1347 (1108–1437)

NOTE. CI, confidence interval.

Tuberculosis control program. The study community is served by a single government primary health care clinic with a dedicated tuberculosis service. All patients with tuberculosis in the community are treated at this facility. The program adhered to the South African National TB Control Program guidelines and included the World Health Organization Directly Observed Treatment Short Course (DOTS) strategy [20]. DOTS coverage in this community was complete, and treatment completion rates for smear-positive disease exceeded 80% [13]. Adult pulmonary sputum-positive tuberculosis was diagnosed on the basis of at least 1 sputum culture positive for *Mycobacterium tuberculosis* or 2 sputum smears containing acid-fast bacilli in the context of a compatible clinical illness. Childhood tuberculosis diagnosis was made with a scoring system using a combination of clinical and radiological features [20]. A score ⩾7 indicated a high likelihood of tuberculosis, using the features length of illness (1–3), nutritional status (1–3), family history of smear-positive disease (3), tuberculin skin test reactivity (3), enlarged lymph nodes (3), abdominal mass (3), central nervous signs (3), chest radiography (3), and spinal angling (4). All sputum testing was performed at the National Health Laboratory Services facilities in Cape Town.

Data sources. Tuberculosis definitions used for notification data were as defined by the South African TB Control Program [20]. Tuberculosis is a notifiable condition in South Africa, and each tuberculosis clinic is required to maintain and report tuberculosis statistics. The numbers of tuberculosis notifications, demographic characteristics, history of previous tuberculosis, sputum microbiologic test findings, and tuberculosis classification data were obtained from the community tuberculosis clinic register. Tuberculosis program data were collected for the years 2004–2008, to cover the period of potential tuberculosis exposure for children aged ⩽5 years in 2008. Demographic data for the community were derived from household censuses performed in 2004, 2006, and 2008 as part of an ongoing health research program. This research was approved by the Research Ethics Committee of the University of Cape Town.

Mathematical transmission model. The number of childhood tuberculosis infections *(C)* within a household with susceptible children *(S)* was assumed to be a function of the number of infectious adults *(I),* their infectivity *(q),* the time of exposure *(t),* the susceptible respiration rate *(p),* and germ-free ventilation *(Q)* as given by the Wells-Riley equation: $C = S(1 - e^{-Iptq/Q})$. The number of infectious adults at any time is given by the smear-positive incidence rate *(M)* and the period of infectivity (Δ). The risk of contact with an infectious adult is given by the Poisson distribution $(\lambda^I/I!)e^{-\lambda}$, where $\lambda = M * A$ is the expected number of infectious adults in a household with A adults. Prevalence was defined as $M/(365/\Delta)$.

Modeled inputs. Germ-free ventilation *(Q)* was calculated as air changes per hour (ACH) for a standard shack dwelling with a volume of 30 m^3. Three values of ACH were modeled: 2 ACH (poor ventilation), 6 ACH (moderate ventilation), and 12 ACH, which is recommended by the World Health Organization for an airborne precaution room [21]. Shacks with closed windows and doors would have an ACH of ⩽2. Shacks with an open window (size, 0.25 M^2) facing the prevailing wind and an open door on the leeward side would achieve 6 ACH with low prevailing wind speeds of 4–5 km/h and 12 ACH with winds of 8–10 km/h [22].

The rate of production of infectious tuberculosis quanta *(q)* was modeled at a value of 1 infectious quantum per hour, which is the mean measured value of smear-positive inpatients in a tuberculosis ward [15]. Sensitivity analyses were performed for values of q between 0.1 and 10 infectious quanta/h. The mean respiratory rate of preschool children aged 0–5 years was estimated to be 225 L/h, which approximates a respiratory volume of 150–200 mL/kg/min [23].

The period of diagnostic delay during which an adult may be infective has been estimated in a systematic review to be very variable but is frequently reported to be 60–90 days [24]. Since Δ may exceed the period of diagnostic delay and is the primary modeled parameter influenced by the functioning of the tuberculosis control program, it was allowed to take values of 30, 60, 90, and 120 days in the model.

For modeling purposes, child time allocation within a 24-h period was 12 h within the home, to allow interaction with resident adults during the evening and night, including 8 h for sleeping and 4 h for other family activities. Similar exposure to nonresident adults can result from adults visiting the primary home or from the child visiting other households. For modeling purposes, 12 h of daytime was allocated to 3 h outdoors, during

which tuberculosis transmission was assumed to be negligible, and 9 h allocated equally between 1–3 additional households with similar numbers of residents as in the primary residence.

RESULTS

Household Survey 2008

The total population of the study community in December 2008 was 14,788, of whom 12,097 were adolescents and adults aged >15 years and 2691 were children aged <15 years, including 1051 children aged <5 years. The total number of households was 6654, of which 1708 contained children aged <15 years and 918 contained children aged <5 years. The age distribution of residents of households with adults only and with both adults and children is shown in Table 1. Crowding in child-containing households was twice as high as that in adult-only households. Of the 918 households with children aged <5 years, 800 contained a single child, 109 contained 2 children, and 9 contained 3 children aged <5 years. The median number of adults in these households was 2.27 per household, and only 28% of these households had ⩾3 resident adults (Figure 1).

Tuberculosis Notifications

From 2004 through 2008, 1289 cases of tuberculosis were notified to the national tuberculosis control program, of which 90% occurred in adults and 10% occurred in children aged ⩽15 years. Of the childhood tuberculosis cases, 66% occurred in children aged <5 years (Table 2). The population increased from 12,803 in 2004 to 14,788 in 2008, resulting in a total of 67,747 person-years of residence. The population growth was restricted to adults, because the population of children aged <5 years remained relatively constant, with 1057 children in 2004 and 1051 children in 2008. A mean of 1.35% of the adult population were identified as having sputum smear–positive tuberculosis each year (Table 2).

Transmission from Resident Adults

Ventilation. The modeled impact of increasing the shack ventilation on the probability of a child becoming infected with tuberculosis is shown for 4 periods of adult infectivity (Δ = 30, 60, 90, and 120 days) in Figure 2*A*. The maximal risk of tuberculosis transmission even under poor environmental ventilation and a prolonged period of adult infectiousness reached only 3%. This maximal condition was primarily determined by the mean number of adults resident in the household and their tuberculosis incidence rate. Transmission could be reduced by a combination of high ventilation and a reduction of the infectivity period. For example, a reduction of the risk of transmission to 1.5% would require either 4, 8, or 12 ACH for Δ values (period of infectiousness) of 30, 60, and 90 days, respectively. Sensitivity analyses with low values of infectious quanta ($q = 0.1$) were unable to reach significant transmission probabilities, and high values of infectious quanta ($q = 10$) reached probabilities >2.75% at all achievable values of Δ and ACH.

Infective period. The modeled impact of increasing periods of adult infectiousness (Δ) on the probability of a child aged <5 years becoming tuberculosis infected is shown for 3 levels of ventilation (2, 6, and 12 ACH) in Figure 2*B*. The benefits of increased ventilation are greatest when Δ is low. Increasing ventilation from 2 to 6 ACH reduces transmission to 2.5% (−16%), to 2.2% (−25%), 1.8% (−36%), and 1.1% (−51%) for Δ values of 120, 90, 60, and 30 days, respectively. Identical reductions in transmission could be alternatively obtained by reducing the child exposure time by 8 h per day. A reduction in exposure time could be achieved by separation of child sleeping areas from those of adults for an 8-h sleeping period. When the modeled infective number of infectious quanta were low ($q = 0.1$), the transmission probabilities of transmission did not reach 1%, and when the number of quanta were high ($q = 10$), the tuberculosis transmission probabilities rapidly became maximal at 3% with minimal sensitivity to either increased ventilation or shortened period of infectivity. The modeled proportions of annual risk of tuberculosis infection due to transmission from resident adults were 70% and 74% for periods of infectivity of 60 and 90 days, respectively. Therefore, we went on to explore additional transmission (26%–30%) that might occur as a result of contact with other potentially infective adults in addition to those residents in the home.

Transmission from Nonresident Adults

The probability of tuberculosis infection as a result of spending 75% of daytime indoors and visiting 1–3 households other than the child's home is shown for visited households with poor ventilation (2 ACH) in Figure 3*A*. Increasing the number of visited households increased the exposure to an additional 2.2 potentially infective adults per household, which resulted in greatly increased probabilities of infection when Δ exceeded 30 days. When multiple poorly ventilated households were visited, annual risks of tuberculosis infection exceeding 4%, 5.5%, and 6% could be achieved when Δ was 60, 90, and 120 days, respectively. Sensitivity analyses of low infectious quanta (q = 0.1) resulted in transmission risks <1%, and high infectious quanta ($q = 10$) resulted in rates of transmission that were directly related to the number of households visited for all modeled values of Δ.

In contrast, the corresponding risks of infection when visiting households with 6 ACH showed minimal increase in transmission risk with increasing number of households visited. Under these moderate ventilation conditions, Δ became the major

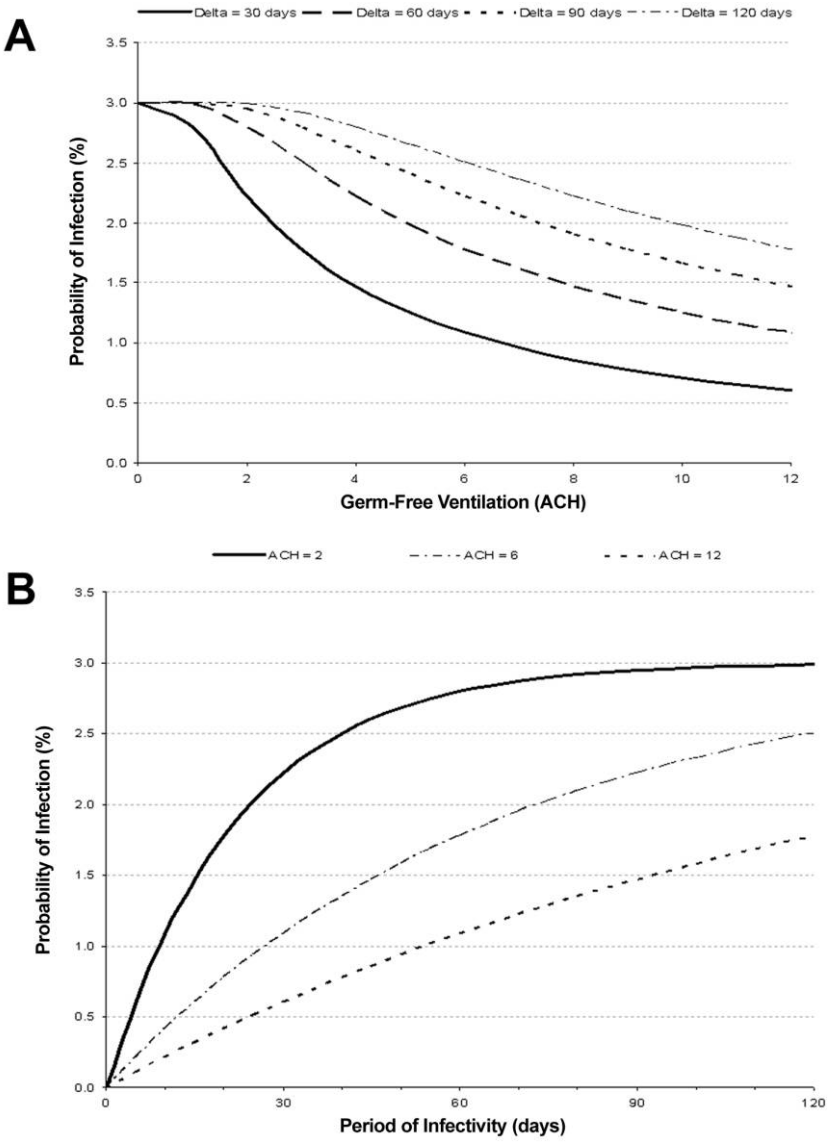

Figure 2. *A,* Effect of ventilation (air changes per hour [ACH]) and mean period of infectivity (delta) on the mean annual risk of tuberculosis infection resulting from a child sleeping in a shack shared with adults. Values are plotted for mean periods of adult infectivity of 30, 60, 90, and 120 days. *B,* Effect of period of infectivity (delta) and ventilation (ACH) on the mean annual risk of tuberculosis infection resulting from a child sleeping in a shack shared with adults. Values are plotted for 2, 6, and 12 ACH. Note that the period of infectivity (delta) is the mean time from onset of infective tuberculosis until initiation of effective antituberculosis chemotherapy. Modeled estimations are based on a potential nighttime exposure of 12 h, a median of 2.2 adult residents per shack, a 1.35% annual risk for smear-positive tuberculosis, and a mean production of 1 infectious airborne quantum of tuberculosis per hour during untreated smear-positive disease.

determinant of tuberculosis transmission risk. Sensitivity analyses of low infectious quanta ($q = 0.1$) resulted in transmission risks of <0.4%, and high infectious quanta ($q = 10$) resulted in rates of transmission of 6%–9% that were directly related to number of households visited for all values of Δ.

DISCUSSION

These modeling analyses demonstrate that the high reported rates of community tuberculosis transmission to children in southern Africa [9–13] can be explained by the interplay between the prevalence of adult infectious tuberculosis, social mixing between adults and children, and the prevailing domestic ventilation characteristics.

The model in this study was based on the Wells-Riley equation, which has been used to examine airborne tuberculosis disease transmission since the 1970s [14] in a wide variety of medical and nonmedical settings and thus has been useful for examining the relative importance of transmission factors in

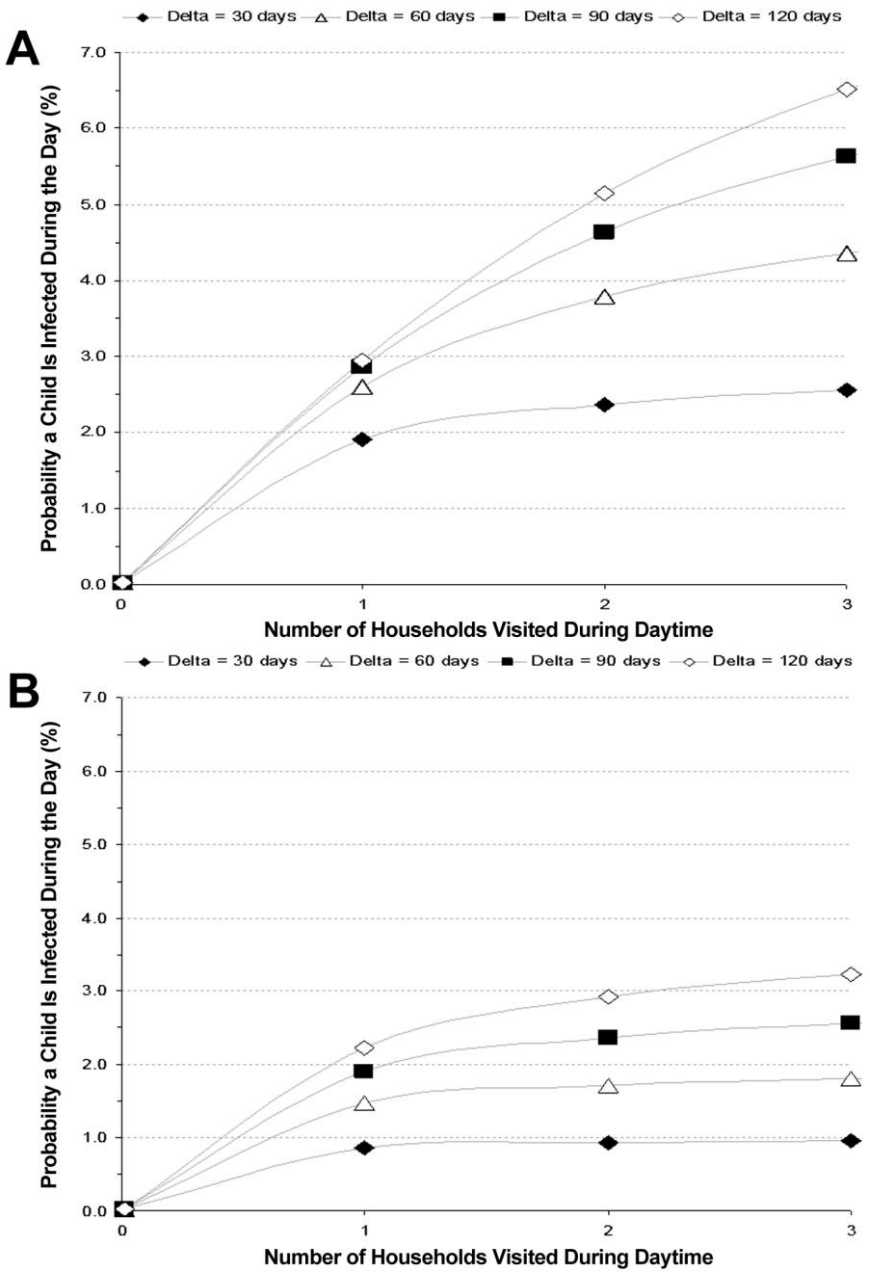

Figure 3. Mean annual risk of tuberculosis infection for a child visiting 1–3 households other than his or her own residential household during the day with ventilation of 2 air changes per hour *(A)* and 6 air changes per hour *(B)*. Values are plotted for mean periods of adult tuberculosis infectivity (delta) of 30, 60, 90, and 120 days. Note that the period of infectivity (delta) is the time from onset of infective tuberculosis until initiation of effective antituberculosis chemotherapy. Modeled estimations are for a preschool child spending 75% of daytime indoors, a median of 2.2 resident adults per visited shack, a 1.35% annual risk for smear-positive tuberculosis, and a mean production of 1 infectious airborne quantum of tuberculosis per hour during untreated smear-positive disease.

real-life situations [15–19]. This study used a novel approach of incorporating population data from a specific South African township to populate the Well-Riley equation, to explore household and nonhousehold tuberculosis transmission to preschool children.

Of particular interest, our model indicated that the potential of the existing clinic-based tuberculosis control program to reduce transmission to children is somewhat limited. Even reductions in Δ (the period of infectiousness) to 30 days by active case finding and rapid tuberculosis diagnosis would have significant impact on transmission to children only when ventilation rates in households exceed 6 ACH. However, such high ventilation rates for informal dwellings during the cold Cape Town winters might be difficult to achieve throughout the year.

Since similar reductions in tuberculosis transmission could be achieved by separating child and adult sleeping areas, this might be a more practicable stratagem.

Another major finding of our study was that a maximum of 75% of the total annual risk of infection could possibly be explained by the interaction between a child and the limited number of adults resident in a primary household. Preschool children are susceptible to tuberculosis infection predominantly because of exposure to infectious adults [6, 7]; therefore, the main determinant of maximal transmission risk in either setting was the number of adults to whom a child was exposed.

Our model also indicated that at least 25% of the risk of infection resulted from exposure to nonresident adults. In well-ventilated settings, transmission was related to Δ, rather than to the number of households visited. In contrast to transmission risks from adult household residents, transmission from nonresident adults can be markedly influenced by the tuberculosis control program's ability to decrease Δ by active case finding. In poorly ventilated nonresidence settings, transmission risks increased markedly with increasing numbers of households visited. These analyses indicate that as children become more socially mobile, the potential for transmission in poorly ventilated nonhousehold settings might become the largest contributor to total transmission risk. Indeed, we have reported increasing tuberculosis infection rates throughout childhood in this community, which peak at ~8% at age 15 years [11].

The strength of this study was the availability of accurate information specific to this community, including the annual risk of tuberculosis infection, the number of adults and children per household, and smear-positive tuberculosis notification rates. A caveat is that some important parameters, such as Δ and the numbers of infective quanta produced by adults with tuberculosis disease, are difficult to measure directly, and estimates were derived from published data. Indeed, Δ may not be identical to the period of diagnostic delay in published studies, and the incidence of smear-positive tuberculosis may only approximate the smear-positive notification rate. The model used the epidemiologic assumption that the tuberculosis epidemic was generalized, with equal mixing of infectivity and contact risks. However, stochastic transmission events, such as close nonhousehold contact with highly infectious individuals, are not captured in this model. Despite these limitations, the outputs from the model were robust and were compatible with the previously observed annual risk of tuberculosis infection in this and similar communities [9–11].

Our findings may give insight to why tuberculosis rates of transmission in South Africa have remained very high despite apparent improvement in case management by the tuberculosis control program [1]. The conditions within crowded African townships with high unemployment rates may have much in common with the conditions present during the industrial revolution of the 18th and 19th centuries, when tuberculosis burdens were also extremely high. Children lived and worked side-by-side with adults [25], but successive factory acts in the United Kingdom and the United States reduced the childhood exposure to adults in the workplace [26, 27]. Improvement in housing and schooling also reduced the amount of close exposures between children and adults. The crèche movement further limited the frequency of contacts between young children and potentially infectious adults [28] and may have the potential to decrease nonhousehold transmission in crowded townships. Reduction in household tuberculosis transmission in poor informal housing will be difficult to achieve. However, improvement of housing stock should particularly focus on improved ventilation and separation of child from adult sleeping areas.

These modeled analyses have identified social and environmental factors that contribute to high rates of tuberculosis transmission in this community. Social mixing patterns of preschool children result in tuberculosis transmission within the extended family, rather than the nuclear family. Where tuberculosis is highly endemic, interruption of community tuberculosis transmission requires prompt treatment of source cases. Tuberculosis control will therefore necessitate an increased focus on active case finding and a reduction in diagnostic delays.

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Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study

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Abstract

Background: Understanding of the transmission dynamics of tuberculosis (TB) in high TB and HIV prevalent settings is required in order to develop effective intervention strategies for TB control. However, there are little data assessing incidence of TB infection in adolescents in these settings.

Methods: We performed a tuberculin skin test (TST) and HIV survey among secondary school learners in a high HIV and TB prevalence community. TST responses to purified protein derivative RT23 were read after 3 days. HIV-infection was assessed using Orasure® collection device and ELISA testing. The results of the HIV-uninfected participants were combined with those from previous surveys among primary school learners in the same community, and force of TB infection was calculated by age.

Results: The age of 820 secondary school participants ranged from 13 to 22 years. 159 participants had participated in the primary school surveys. At a 10 mm cut-off, prevalence of TB infection among HIV-uninfected and first time participants, was 54% (n = 334/620). HIV prevalence was 5% (n = 40/816). HIV infection was not significantly associated with TST positivity (p = 0.07). In the combined survey dataset, TB prevalence was 45% (n = 645/1451), and was associated with increasing age and male gender. Force of infection increased with age, from 3% to 7.3% in adolescents ≥20 years of age.

Conclusions: We show a high force of infection among adolescents, positively associated with increasing age. We postulate this is due to increased social contact with infectious TB cases. Control of the TB epidemic in this setting will require reducing the force of infection.

Background

Tuberculosis (TB) remains a major cause of morbidity and mortality in the world[1]. In order to develop effective intervention strategies for TB control, it is important to understand TB transmission in high burden settings. While there have been recent studies assessing TB infection in young children[2-5], there are few data assessing TB infection in older children and adolescents in communities with high TB and HIV burdens[6].

Incidence of TB infection is a measure of current transmission in a community. While repeated testing of uninfected individuals over time is a conventional method for determining incidence of a disease, this methodology is both labour and time intensive, and is further complicated by the boosting of the immune response in immunology-based tests such as the tuberculin skin test (TST)[7,8]. Therefore alternative approaches for calculating incidence from prevalence data have been developed. The annual risk of TB infection (ARTI), calculated from TB infection prevalence data, is an averaged measure of risk of TB infection over the lifetime of the study participants[7,9]. The limitation of this measure is that ARTI only provides an estimate of current transmission or incidence if calculated in very young participants. In comparison, force of infection, defined as the proportion of susceptible individuals that have become infected

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with *Mycobacterium tuberculosis* in a specified period, can be calculated using changes in age-specific prevalence rates[10-12], and provides an estimate of recent infection incidence across a wider age range.

This approach is a relatively new concept that has been utilized to estimate incidence in diseases in which true incidence is difficult or costly to measure, such as glaucoma[13] and more recently, HIV[12,14]. The mathematical techniques utilized are based on the principle that prevalence is a function of incidence and duration of illness or infectivity[15]. This principle holds true under the assumptions of disease stability. Data from this community suggest that TB transmission has remained relatively constant over the past decade, as evidenced by the stable TB notification rates among HIV-uninfected adults from 1997 to 2008[16], as well as the stable childhood TB over the same time period[2].

We performed a TST survey among adolescents in a high HIV and TB prevalence community to assess prevalence of TB infection and force of infection by age.

Methods

This study was the third in a series of cross-sectional tuberculin skin testing surveys performed among school-attending children in the study community. The first two surveys were performed in the local primary school in 2006 and 2007[2]. The survey reported in this manuscript was performed in children attending secondary school in the study community in 2009. The same methodology was used in all three surveys, with the addition of HIV testing in the secondary school survey. Children were eligible if they were resident in the community and registered at the local secondary school. Parental consent, and assent from participants <18 years of age, were obtained prior to enrolment. Adolescents ≥18 years of age provided written consent. Basic demographic information was collected and participants were examined for the presence of a BCG scar. Participants received the TST regardless of BCG scar status.

The WHO-recommended standard Mantoux test of 2TU of PPD (Purified Protein Derivative) RT23 with Tween 80 (Statens Seruminstitut, Copenhagen) was administered intradermally to the volar surface of the left forearm by a trained nurse. The tuberculin reaction size was read by a trained assessor three days following the inoculation. The presence or absence of a reaction was noted, and, where present, the size of the induration was measured along perpendicular axes using standard calipers. Participants also provided an oral transudate specimen for HIV testing, using the Orasure® collection device and Vironostika Uni-Form II HIV-1 and HIV-2 plus 0 ELISA test (bioMérieux SA, Marcy l'Etoile, France). HIV results were anonymous but linked to TST results and all adolescents were encouraged to have separate voluntary counseling and testing at local facilities. HIV testing was not performed in the primary school surveys[2].

This study was approved by the University of Cape Town's Research Ethics Committee. All children with a TST reaction ≥10 mm were recalled for investigation for active tuberculosis and referred to the local clinic for further management if appropriate.

Data were analyzed using STATA 9.0 (StataCorp, College Station, Texas). Analysis was performed in two parts: firstly on the secondary school dataset, excluding HIV-infected individuals, and secondly on the secondary school dataset combined with the primary school dataset, excluding the HIV-infected individuals from the secondary school survey. Children who declined HIV testing were considered to be HIV-uninfected for the purposes of the TB analysis.

TST results were calculated as the mean of the two diameters of the TST reaction: a positive reaction was defined at 10 mm cut-off, based on clinical guidelines[17,18]. ARTI was calculated as $1\text{-}(1\text{-prevalence})^{1/(\text{mean age}+0.5)}$[19]. As the age in full years at participants' last birthday was used, 0.5 was added to the mean age for the calculation of ARTI[19]. The secondary school sample was divided into age quartiles and TB prevalence and ARTI were calculated overall and for each age group. Bivariate analyses employed Student's t-, Fisher's exact and Wilcoxon sum rank tests, as appropriate. Multiple logistic regression models were developed to examine factors associated with positive TST results.

HIV data secondary school participants

Univariate and multivariate logistic regression models were developed to determine the demographic characteristics associated with HIV, and to assess for an association between HIV and TB infection. In these models, a TST reaction size of ≥5 mm was used as a positive cut-off for TB infection in HIV-infected adolescents, in keeping with clinical guidelines[17,18].

Subset of repeat TST participants

Overall, 159 students who had participated in the 2007 survey also took part in the secondary school survey, resulting in repeat tuberculin testing of these participants. Repeated TST tests may be associated with boosting of the immune response, complicating the interpretation of the second TST reaction size[7,8]. We, therefore, analyzed this subset of participants separately from the rest of the cohort. In keeping with existing literature, we defined a true conversion to TST positivity between the two surveys as a change from a negative result (<10 mm) on original TST to a positive result (≥10 mm) on the second test, with an absolute reaction size increase of at least 6 mm[20-22]. Bivariate analyses

used Wilcoxon sum rank and Student's t tests, as appropriate for comparison of participants with repeated TST compared to first time participants in 2009. A multivariate regression model was developed to compare reaction sizes between the two groups. The McNemar test was used for matched comparison of 2007 and 2009 TST results.

Combined surveys database

In order to assess the effect of age on TB prevalence and force of infection, we combined the primary and secondary survey datasets, excluding HIV-infected participants from 2009, as well as the second test in those participants who had repeat tests in 2009. The participants were divided into age quartiles and TB prevalence and ARTI were calculated overall and for each age group.

Smoothed prevalence of TB infection by age was calculated from predictive logistic regression models on the combined primary and secondary school dataset, overall and stratified by gender, excluding those who tested HIV-infected in the secondary school. Force of infection was calculated at specific ages for the pool of individuals who remained uninfected [annual change in prevalence/(1-prevalence)]. Trends in ARTI and force of infection were assessed using Cox-Stuart test for trend [23,24].

For all analysis, 95% confidence intervals (CI) were based on the Poisson distribution and all statistical tests were 2-sided at alpha = 0.05.

Results

Of the 959 children enrolled in the secondary school, 839 were eligible for study participation (87%). Ineligibility was due to residence outside of the community (n = 80) or having dropped out of school (n = 40). Consent/assent was obtained for 820 children (98% of those eligible). Refusal by parent or learner accounted for 18 non-consenters and 1 child was not consented due to absenteeism over the study period. All 820 consented students were enrolled. Four of the children enrolled declined tuberculin skin testing, and four children declined HIV testing. Of the 816 children who underwent skin testing, 813 (99.6%) had the TST reaction read within 72-96 hours (Figure 1). No study-related adverse events were noted in the three children assessed outside the window period and these participants were excluded from the analysis.

Table 1 shows the demographic characteristics of the secondary school study cohort. Of the 813 participants who completed TST testing, 159 had received tuberculin skin testing in the 2007 survey in the community. These participants were excluded from the main analysis and their results are presented in a sub-analysis. Of the remaining 654 children, all but three of the participants underwent HIV testing. In total 34 participants of the remaining participants tested HIV-infected, and were excluded from the TB analysis (n = 620; Figure 1).

Among the 620 participants, ages ranged from 13 to 22 years, with a mean age of 17.5 years, and 59% of participants were female. The majority of the children did not have a BCG scar (87%); one child's BCG scar status was not recorded, and this participant was excluded from analysis involving BCG scar status.

TST reaction sizes ranged from 0 to 30 mm (median = 11.5 mm; IQR: 0-16.5 mm), and 222 children had a TST result of 0 mm (36%). The frequency distribution of reaction sizes >0 mm are presented in Figure 2. There was no significant difference between the median size of reactions in participants with BCG scars compared to those without scars (11 vs 11.5 mm respectively, p = 0.24).

At the 10 mm cut-off 334 participants (54%) had a positive TST result. There was no significant difference in the defined TB positivity by BCG scar status (p = 0.99). In a multivariate logistic regression model predicting the relative odds of a positive TST result, age was positively associated with a positive TST result (adjusted odds ratio (OR) for a 1-year increase in age: 1.10, 95% CI: 1.01 - 1.21; p = 0.03), as was male gender (adjusted OR for female compared to male: 0.65, 95% CI: 0.47-0.90; p = 0.01).

The overall ARTI for this sample was 4.2% (95% CI: 3.8-4.7%). Table 2 reports TB prevalence and ARTI overall, and by age quartiles. The ARTI did not differ significantly across the age quartiles (p = 0.75) or across age by years from 14 to 21 years of age (p = 0.63).

Sub-analysis: HIV and TB infection

Of the 820 participants enrolled in the secondary school survey, 816 (99.5%) consented to HIV testing. In total 40 (4.9%) participants tested HIV-positive. In a logistic regression model, HIV infection was associated with increasing age (OR: 1.3; 95% CI: 1.1-1.6; p = 0.001) and female gender (OR: 2.3; 95% CI: 1.1-4.7; p = 0.03).

Among the 809 participants who underwent both TST and HIV testing, 38 were HIV-infected, four of whom had previous TST testing in 2007. The median TST reaction size among HIV-infected participants (amongst those who did not have repeat TST assessment; n = 34) was smaller than that of HIV-uninfected participants (0 vs 11.5 mm; p = 0.08), and this difference was significant when adjusted for age and gender (p = 0.04). Using the revised cut-off of ≥5 mm, 15 (40%) HIV-infected participants were TST positive. In a multivariate logistic regression model adjusted for age and gender, HIV infection was not significantly associated with TST positivity (OR = 0.53; 95% CI: 0.27-1.05; p = 0.07).

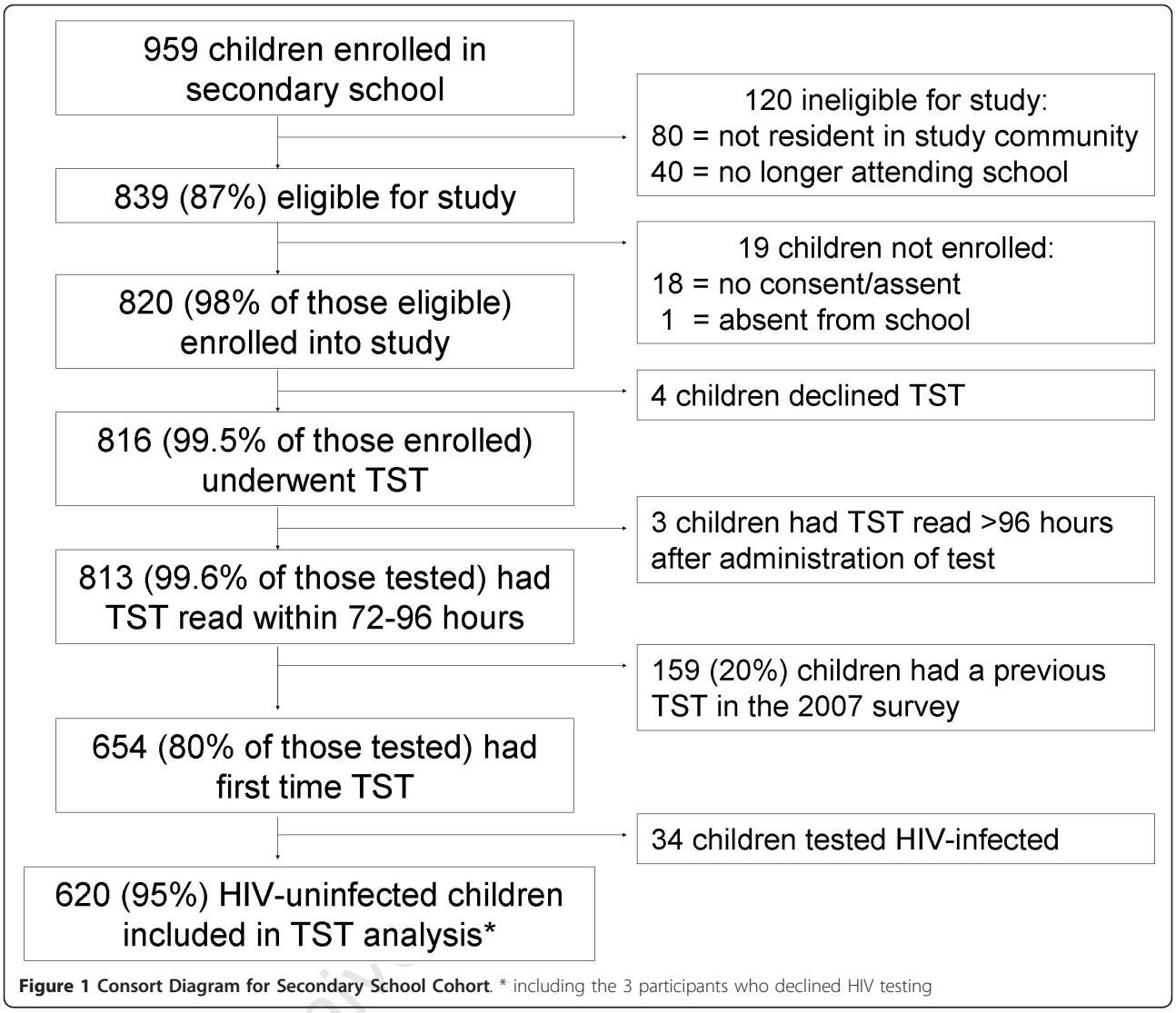

Figure 1 Consort Diagram for Secondary School Cohort. * including the 3 participants who declined HIV testing

Table 1 Demographic characteristics of the secondary school sample.

	First time TST participants[Φ]			Repeat TST participants[ΦΦ]		
	HIV-uninfected	HIV-infected	Total	HIV-uninfected	HIV-infected	Total
	n = 617	n = 34	n = 654	n = 154	n = 4	n = 159
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Age: mean (range)	17.5 (13-22)	18.4 (15-22)	**17.5 (13-22)**	15.4 (13-19)	15.3 (14-17)	**15.4 (13-19)**
13-16 yrs	176 (29)	5 (15)	**183 (28)**	133 (86)	3 (75)	**136 (86)**
17 yrs	148 (24)	4 (12)	**152 (23)**	13 (8)	1 (25)	**15 (9)**
18 yrs	112 (18)	9 (26)	**121 (19)**	6 (4)	0 (0)	**6 (4)**
19-22 yrs	181 (29)	16 (47)	**202 (30)**	2 (1)	0 (0)	**2 (1)**
Gender: Male	255 (41)	9 (34)	**266 (41)**	81 (53)	2 (50)	**84 (53)**
BCG Scar present	80 (13)	6 (18)	**86* (13)**	32 (21)	0 (0)	**32 (20)**
TST positive	331 (54)	11 (32)	**345 (53)**	90 (58)	1 (25)	**91 (57)**

*n = 653: one participant's BCG scar status was not recorded

[Φ] Three participants declined HIV testing

[ΦΦ] One participant declined HIV testing

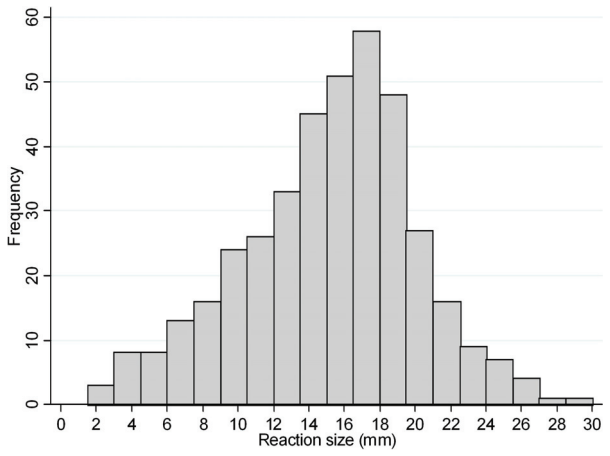

Figure 2 Frequency distribution of TST reactions (>0 mm) in HIV-uninfected participants in secondary school survey.

Sub-analysis: Repeated TST testing

In 2009, 159 students who had participated in the 2007 survey took part in the secondary school survey, four of whom were HIV-infected. The mean reaction size in 2009 among participants with repeat tests was larger compared to participants who tested for the first time (15 vs 11.5 mm; $p < 0.001$), and this finding persisted when adjusted for age and gender ($p < 0.001$). Overall, TB prevalence did not differ between those that had a repeat TST test and those participants who were testing for the first time (58 vs 54%, $p = 0.29$).

Using a 10 mm cut-off for positivity, in 2007, 76 (48%) of the 159 participants had a positive TST result; in 2009 91 (57%) tested TST positive. Overall, 19 of the 83 participants who were TST negative in 2007 tested positive in 2009 ($p = 0.003$), and four (5%) reverted to a TST negative result. Using the definition outlined above, 18 of the 19 convertors had an increase in reaction size of ≥6 mm and were therefore considered true new infections with TB. These 18 participants, from the pool of 83 susceptible children in 2007, equate to an incidence of 22% over two years (95 CI: 13-32%), or an annual incidence of infection of 11% ([18/83]/2).

Table 2 Secondary school TB prevalence and ARTI by age quartile for the 10 mm cut-off point (excluding HIV-infected and repeat TST participants):

Age Category	Mean Age*	n (%)	TST positive	Prevalence	ARTI% (95% CI)
13-16 yrs	15.8	178 (29)	85	47.8%	4.0% (3.2-5.0%)
17 yrs	17.5	148 (24)	76	51.4%	4.0% (3.2-5.0%)
18 yrs	18.5	112 (18)	58	51.8%	3.9% (2.9-5.0%)
19-22 yrs	20.2	182 (29)	115	63.2%	4.8% (4.0-5.8%)
TOTAL	**18**	**620**	**334**	**53.9%**	**4.2% (3.8-4.7%)**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section.

Note: Participants who declined HIV testing were presumed to be HIV-uninfected and were included in the TB analysis

Combined Primary and Secondary school database

In order to assess the effect of age on TB prevalence and force of infection, we investigated combining the primary and secondary school surveys. We compared the prevalence of TST positive results for the three cohorts in the overlapping age ranges of 10 to 12 years and 14 to 16 years. The chi-squared test for comparison was not significant (survey 1 and 2: $p = 0.66$; survey 2 and 3: $p = 0.46$), nor was survey year a significant risk factor in multivariate regression model for TST positivity ($p = 0.23$). Therefore we combined these two datasets. In the primary school survey, TST readings were available for 831 of the 832 children enrolled. Therefore the combined database, excluding the participants who tested HIV-infected, was comprised of 1,451 participants.

Ages of the combined cohort ranged from 5 to 22 years, with a mean age of 13.6 years (standard deviation = 4.1), and 52% of participants were female. The majority of the children did not have a BCG scar (80%); BCG scar status was not available for two participants and they were therefore excluded from analysis involving BCG scar status.

TST reaction sizes ranged from 0 to 30 mm (mean = 8.0; median = 0 mm). Overall 728 (50%) of the participants had no reaction to the TST. At the 10 mm cut-off 645 participants (45%) had a positive TST result (Table 3). TB prevalence by age is presented in Figure 3. In a multivariate logistic regression model a positive TST result was significantly associated with age (adjusted OR for a 1-year increase in age: 1.17, 95% CI: 1.10 - 1.25; $p < 0.001$) and male gender (adjusted OR for female compared to male: 0.74, 95% CI: 0.60-0.92; $p = 0.01$). TST positivity was not associated with BCG scar status ($p = 0.40$).

Table 4 reports the smoothed prevalence of TB infection by age, as predicted by our logistic regression model. Also reported in Table 4 are the ARTI and force of infection by age, calculated from the smoothed prevalence. The overall ARTI for this sample was 4.1% (95% CI: 3.8-4.4%). The ARTI did not differ significantly across age by years from 6 to 22 years of age ($p = 0.15$). The force of infection increased with increasing age, and this trend was significant ($p = 0.01$).

Discussion

This is one of the first studies to report TB infection prevalence, ARTI and force of infection in adolescents in a high TB and HIV prevalent setting.

Table 3 TB prevalence and ARTI by age quartile for the 10 mm cut-off point for the three surveys combined (excluding HIV-infected and second tests in repeat TST participants):

Age Category	Mean Age*	n (%)	TST positive	Prevalence	ARTI% (95% CI)
Age Category					
5-9 yrs	8.3	325 (22)	91	28.0%	3.9% (3.1-4.7%)
10-14 yrs	12.8	488 (34)	207	42.4%	4.0% (3.2-5.0%)
15-17 yrs	16.7	344 (24)	174	50.6%	3.9% (2.9-5.0%)
18-22 yrs	19.6	294 (20)	173	58.8%	4.8% (4.0-5.8%)
Gender					
Male	13.8	691 (48)	330	47.8%	4.6% (4.1-5.1%)
Female	14.3	760 (52)	315	41.4%	3.7% (3.3-4.1%)
TOTAL	**14.1**	**1,451**	**645**	**44.5%**	**4.1% (3.8-4.4%)**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section.

Note: Participants who declined HIV testing were presumed to be HIV-uninfected and were included in the TB analysis

In this study we confirmed the high ARTI rate reported in the primary school children[2], and show a high force of infection. ARTI provides a measure of the averaged risk of infection over the participants' lifetime. Therefore this measure pertains to the annual risk over a period of up to 20 years, and consequently the ARTI provides little information on the current transmission within the study population.

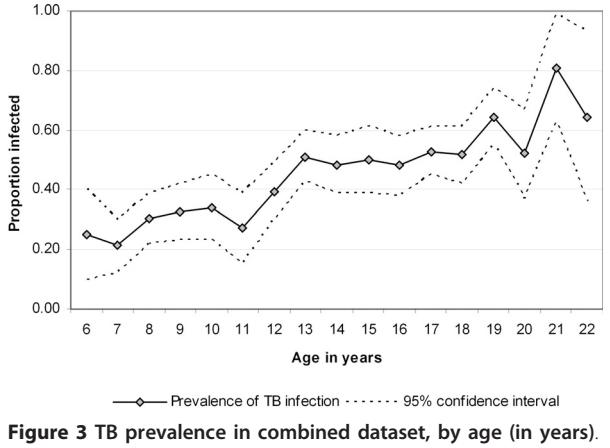

Figure 3 TB prevalence in combined dataset, by age (in years). Note: This figure is based on the unadjusted data presented in Table 3.

Force of infection is a measure of recent transmission and the high rates reported here are in keeping with the substantial burden of TB prevalence[25] and notifications[26] in this community and in South Africa[1]. We demonstrated that force of infection was positively associated with increasing age. These findings are consistent with those reported prior to the HIV epidemic[27], as well as by more recent mathematical modeling[6]. The advantage that this study had over the modeling paper is that of greater numbers of older adolescent participants all recruited from the same community, and the ability to show that the force of infection continues to increase up to approximately 20 years of age. In adulthood force of infection becomes harder to measure due to a reduced proportion of susceptible individuals, and the inability to identify secondary infections.

Force of infection is a function of the probability of an effective encounter with an infectious TB case, and as such is a product of TB disease prevalence and mixing patterns. We postulate that the association between force of infection and age may be due to increasing social mixing patterns, resulting from changing social interactions associated with age. We have previously shown that TB infection in primary school children is associated with an adult TB case on their residential plot[28], while TB transmission between adults is due to social interactions off the residential plots[6]. Data from Europe shows that number of social contacts peak in adolescents[29], suggesting that the likelihood of contact with infectious persons may also peak at this time. The social interactions, and therefore risk of TB infection, of mid-teens may more closely resemble that of adults rather than younger children in the community.

We have also shown a changing risk of TB infection by gender: in the primary school surveys, gender was not associated with TB infection[2], but in the secondary school survey, male gender was associated with increased TB infection. As males get older, their risk of TB infection out-strips that of females of similar age, and this is consistent with other reports in the literature [9,21]. This increased risk may be due to a combination of increased biological susceptibility, differing immunological responses or differences in socialization patterns of male compared to female adolescents.

The lack of association between HIV and TB infection is a key finding: adolescents infected with HIV did not appear to be at higher risk for acquiring primary TB infection. This is in keeping with findings reported from lower HIV and TB prevalent settings[30,31], and consistent with studies that suggest the establishment of TB infection is mechanistic, as opposed to immune-based [32]. However, adolescents are at increased risk of progression to TB disease in the first two years following TB

Table 4 Predicted prevalence, ARTI and force of infection by age in study cohort

Age	Number of participants	Predicted Prevalence* (95% CI)	ARTI**	Force of infection $^{\Phi}$
5	1	0.22 (0.17 - 0.27)	4.85	
6	36	0.24 (0.19 - 0.29)	4.47	2.93
7	84	0.26 (0.22 - 0.30)	4.21	3.15
8	112	0.28 (0.24 - 0.32)	4.06	3.39
9	92	0.31 (0.27 - 0.34)	3.96	3.81
10	71	0.33 (0.29 - 0.36)	3.91	3.96
11	59	0.35 (0.32 - 0.39)	3.89	4.28
12	107	0.38 (0.35 - 0.41)	3.91	4.47
13	135	0.41 (0.38 - 0.43)	3.93	5.03
14	116	0.43 (0.41 - 0.46)	3.97	5.11
15	84	0.46 (0.44 - 0.49)	4.04	5.58
16	106	0.49 (0.46 - 0.52)	4.10	5.70
17	154	0.52 (0.48 - 0.55)	4.18	6.05
18	112	0.54 (0.51 - 0.58)	4.27	6.44
19	101	0.57 (0.53 - 0.61)	4.36	6.63
20	46	0.60 (0.55 - 0.64)	4.45	7.11
21	21	0.62 (0.58 - 0.67)	4.55	7.37
22	14	0.65 (0.60 - 0.70)	4.66	7.34

* Predictive logistic regression model: logit{P(Age)} = -1.82+0.111 age.

** The ARTI calculations are based on the mean age for the age category+0.5 years, as described in the methods section.

$^{\Phi}$ Force of infection = annual change in prevalence/(1-prevalence)

infection[33], and the high infection rates in this community place adolescents at substantial risk of TB disease.

The high incidence rate of TB infection in the subset of participants tested 2 years apart, confirms the high force of infection. However, due to the boosting of the immune response with enhanced allergy noted with repeated TSTs[8,34-38], the incident rate in this subset is higher than the force of infection. This is highlighted by the larger median TST reaction sizes in participants with a repeat test compared to first time participants. Similarly, reduced and anergic responses to TST have been noted in HIV-infected patients[39-42], as evidenced by the smaller median reaction sizes in HIV-infected participants compared to HIV-uninfected participants. These two scenarios highlight a limitation of using an immune-based test for determining TB infection.

Only 20% of the children in this study had an observable BCG scar, despite the South African policy to vaccinate all infants with the BCG vaccine[43]. However BCG scarring may be variable[38,44] and we found no difference in the distribution of TST results between those children with or without BCG scars.

In this study, only 1% of TST results were weakly positive (1 to 5 mm), suggesting minimal cross reaction with environmental mycobacteria[19]. Given the high TB prevalence in the sample, the positive predictive value of the TST is likely to be high.

HIV testing was not performed in the primary school surveys. However, the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model for the African population[45,46] reports an HIV prevalence of <4% in this age group. Therefore HIV is unlikely to have substantially impacted TST readings in this group and, given the anergic reactions to TST associated with HIV[39-42], any impact of HIV is likely to result in an underestimate of TB infection. We were able to exclude HIV-infected children from the secondary school TB analysis, thereby reducing the potential bias resulting from dual infections.

This study had a very low refusal rate (<4% across all three surveys), and as such volunteerism is unlikely to have biased our results. However, it should be noted that the school-attending children may not be representative of all the children in the community, in particular with regards to risk of HIV infection[47,48]. However, should either HIV or TB infection be higher among non-school attending children, this would result in an underestimation of the prevalence and force of TB infection in this study.

The high force of infection in this community would result in significant rates of primary and secondary TB infection and, given the high HIV prevalence among adults[6], and the considerable increased risk of progression to TB disease in HIV-infected individuals[30,49],

these findings could explain the substantial incidence of TB disease in this setting. Control of the TB epidemic requires an increasing proportion of non-infected individuals in a population, in other words, a decreased force of infection[50]. In order to reduce the force of infection, National Tuberculosis Programmes need to decrease the prevalence of infectious cases in the community. We have previously shown that a high coverage antiretroviral treatment (ART) program will reduce TB prevalence among HIV-infected participants, due to both improved immune function and the active TB case-finding among patients initiating ART[25]. Extending active case finding to HIV-uninfected residents may substantially reduce the burden of infectious cases. Social programs, such as improved housing, that impact the environment in which individuals interact may also lead to a reduction in the incidence of TB infection.

Conclusions

In conclusion, these data suggest a substantial force of infection among adolescents, which is associated with increasing age. This is most likely due to changing social mixing patterns among adolescents, resulting in increased contact with infectious TB cases. Studies into social interaction patterns at different ages in this setting may help to better understand this increasing risk of TB infection. HIV infection was not associated with increased risk of TB infection. However, the extremely high force of infection, together with the high community HIV prevalence, explains the overwhelming burden of TB disease in this township. Control of the TB epidemic will require reducing the force of infection, and further studies assessing intervention strategies such as those suggested here are required.

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Authors' contributions

KM, LGB, RW, LM and LDHA designed the study. KM, LDHA and ES collected the data. KM did the analyses with input from RW, LM and HL. KM wrote the paper with input from all the authors who each approved the final version.

Competing interests

The authors declare that they have no competing interests.

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